2008

THE EFFECTS OF LOBELINE ON METHAMPHETAMINE-INDUCED CONDITIONED PLACE PREFERENCE AND DOPAMINERGIC ALTERATIONS IN THE NUCLEUS ACCUMBENS SHELL

Nichole Marie Neugebauer
University of Kentucky, nneug2@uky.edu

ABSTRACT OF DISSERTATION

Nichole Marie Neugebauer

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Arts and Science at the University of Kentucky

By
Nichole Marie Neugebauer

Lexington, Kentucky

Director: Dr. Michael T. Bardo, Professor of Psychology

Lexington, KY

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Previous research has suggested that lobeline, a plant alkaloid derived from *Lobelia inflate*, has potential to be an efficacious pharmacotherapy for the treatment of methamphetamine dependence. In addition to attenuating methamphetamine-induced dopaminergic alterations *in vitro*, lobeline has been shown to decrease the primary rewarding effects and discriminative stimulus properties of methamphetamine in rats. It is of clinical interest to assess the utility of lobeline to decrease methamphetamine conditioned cues as these cues have been shown to significantly contribute to relapse.

The current studies assessed the ability of lobeline to block the acquisition and expression of methamphetamine-induced conditioned place preference in rats. Lobeline blocked the acquisition of methamphetamine-induced conditioned place preference when a low dose of methamphetamine was used during conditioning. However, this blockade was surmounted with higher doses of methamphetamine. Furthermore, the expression of methamphetamine-induced conditioned place preference is attenuated following repeated administration, indicating that lobeline not only blocks the primary reinforcing effects of methamphetamine, but it also blocks the environmental cues that become associated with drug administration. These results provide further evidence that lobeline may be an efficacious treatment for methamphetamine dependence.

The rewarding properties of psychostimulants are thought to be mediated, at least in part, by the nucleus accumbens shell. The effects of lobeline on methamphetamine-induced alterations in this dopaminergic region were assessed using microdialysis in rats. Acute lobeline did not have an effect on the methamphetamine-induced increases in dopamine, indicating that repeated lobeline administration may be more efficacious. Interestingly, lobeline potentiated the methamphetamine-induced decrease of the dopamine metabolite, DOPAC. These results suggest that acute lobeline may function to redistribute vesicular dopamine pools within the terminal bouton.

KEYWORDS: Methamphetamine, Lobeline, Conditioned Place Preference, Microdialysis, Nucleus Accumbens Shell

Nichole Marie Neugebauer
Student Signature

07/16/2008
Date

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By

Nichole Marie Neugebauer

Dr. Michael T. Bardo
Director of Dissertation

Dr. David Berry
Director of Graduate Studies

07/16/2008

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ACKNOWLEDGEMENTS

The following dissertation, while an individual work, benefited from the insights and direction of several people. First, my Dissertation Chair, Dr. Michael T. Bardo, exemplifies the high quality scholarship to which I aspire. In addition, Dr. Steven B. Harrod provided instructive comments and engaged in many discussions which have contributed to this project. Next, I wish to thank the complete Dissertation Committee, and outside reader, respectively: Dr. Chana Akins, Dr. Linda Dwoskin, Dr. Mark Prendergast, and Dr. Wayne Cass. Each individual provided insights that guided and challenged my thinking, substantially improving the finished product.

In addition to the technical and instrumental assistance above, I received equally important support from my family and friends. Without your ongoing love and support during this process, completion of this project would not have been possible.

This research was funded by NIDA grant R01 DA13519. For purposes of full disclosure, the University of Kentucky holds patents on lobeline and lobelane, which have been licensed by Yaupon Therapeutics, Inc. (Lexington, KY). A potential royalty stream to Linda P. Dwoskin, PhD and Peter A. Crooks, PhD may occur consistent with University of Kentucky policy, and both L.P.D. and P.A.C. are founders of, and have financial interest in, Yaupon Therapeutics, Inc.

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CHAPTER ONE
Introduction

Methamphetamine Dependence: Current Trends and Treatments.

The negative impact that methamphetamine availability has had on our society is far-reaching. Methamphetamine is commonly synthesized in clandestine laboratories using extremely caustic chemicals, which can readily create dangerous situations including environmental contamination, explosions, and fires. Mere exposure to these laboratories can result in negative health consequences. However, the health consequences apparent in individuals that habitually use methamphetamine can be devastating as these individuals are at increased risk for unsafe sexual behaviors, cardiovascular problems, convulsions, and potentially long-lasting psychotic behavior (NIDA, 2007b).

Despite efforts to curb methamphetamine production and use, a 2007 telephone survey of 500 county law enforcement officers (sheriffs) from 43 states indicated that 47.5% of the sheriffs surveyed reported methamphetamine as the most problematic drug of abuse in their county (NACO, 2007). While the number of methamphetamine laboratories seized by officials peaked in 2003 and has steadily decreased in recent years, the availability of methamphetamine has not decreased as evidenced by 80% of the sheriffs surveyed reporting that the availability of the drug has remained the same or increased in the last year (DEA; NACO, 2007). Although the stereotypic methamphetamine abuser is a white male between 18 and 30 yrs old, an increased number of adolescents, women and ethnic minorities are using methamphetamine (NACO, 2007). Additionally, recent reports from NIDA's 2006 Monitoring the Future Survey indicate that crystal methamphetamine use among young adults has not decreased over the last four years (NIDA, 2007a). These epidemiological data illustrate the persistent use of methamphetamine in the United States.

Currently, there are few options available for treating methamphetamine dependence. Participation in behavioral therapies such as contingency management programs have been shown to promote abstinence above that of standard or no treatment (Prendergast, Podus, Finney, Greenwell, & Roll, 2006; Roll et al., 2006; Shoptaw et al., 2005). In addition to behavioral therapies, efforts

have been underway to identify an efficacious pharmacotherapy to aid in treating methamphetamine dependence. A recent review highlights possible approaches that may be utilized in the development of a pharmacotherapy including immunotherapy and novel medications that would alter methamphetamine pharmacodynamics (Vocci & Appel, 2007). Potential therapeutic targets include the vesicular monoamine transporter, the dopamine transporter, dopamine receptors, as well as GABA(Gamma-aminobutyric acid)ergic, glutamatergic, serotinergic, endogenous opioid, and endocannabinoid pathways (Vocci & Appel, 2007). Identification of a useful pharmacotherapy to aid in treating physiological alterations that result from methamphetamine dependence may allow for better outcomes following behavioral therapies (Ling, Rawson, Shoptaw, & Ling, 2006). Research efforts to identify and develop an efficacious pharmacological aid for the treatment of methamphetamine dependence are ongoing.

Pharmacokinetics of Methamphetamine

Rodent models are often employed to study various neurochemical and behavioral aspects of psychoactive substance administration and have afforded an improved understanding of potential mechanisms underlying the rewarding effects of methamphetamine. In rodent models, psychoactive drugs are typically administered intravenously, subcutaneously or intraperitoneally. While humans administer methamphetamine intravenously, it is also commonly administered intranasally and by inhalation of the smoke that results from heating it. In rats, the plasma concentration of methamphetamine (3 mg/kg) reaches a maximum level faster following intraperitoneal (5 – 10 min) administration than following subcutaneous administration (20 – 30 min); however, ~42 % of methamphetamine administered via intraperitoneal injection is subject to first-pass metabolism (bioavailability ~52 %), whereas the bioavailability is 100% following intravenous and subcutaneous injection (Gentry et al., 2004). While intravenous administration provides 100% bioavailability in humans, the bioavailability of methamphetamine is lower with other routes of administration, such as intranasal (79%) and smoked (67%; Harris et al., 2003). Following a single administration of methamphetamine that results in equivalent peak plasma concentrations in rat and human, the elimination half-life is ~70 min and ~ 12 hr, respectively (Cho, Melega, Kuczenski, &

Segal, 2001; Cook et al., 1993). Although most of the information available on methamphetamine pharmacokinetics is derived from male subjects, there is evidence that there are sex differences in methamphetamine serum levels achieved following intravenous administration in rats; further characterizations of sex differences with regard to pharmacokinetics warrants further investigation given the increased use among women (Milesi-Halle, Hendrickson, Laurenzana, Gentry, & Owens, 2005). Differences in the bioavailability of a methamphetamine across routes of administration and the faster elimination of drug in rodents compared to human should be taken into careful consideration when evaluating findings from preclinical research.

Mechanisms of Drug Dependence

Theoretical Framework

The processes involved in learning and memory make it possible for an individual to survive in their environment. One theoretical framework for drug addiction suggests that the neurochemical consequences of psychoactive drug administration may result in maladaptive learning. Associations learned during repeated drug administration result in a distinct behavioral pattern involving an incredibly high motivation to obtain drugs and an inability to abstain from administering them, despite health and social consequences (Di Chiara, 1999; Hyman & Malenka, 2001). There are several dissociable types of learning that are affected by repeated exposure to psychostimulants, including associative (instrumental and classical conditioning), non-associative (habituation and sensitization), and procedural (skills and habits). Many of these learning processes rely on the same neurobiological mechanisms that are altered following exposure to psychoactive substances. It has been suggested that the milieu of drug-induced neurobiological alterations ultimately results in abnormal, maladaptive reward-related learning (Kelley, 2004). Investigating the interactions of psychostimulant-induced neurobiological changes and reward learning may afford an efficacious treatment for drug dependence that includes synergistic behavioral and pharmacological interventions.

Neuroanatomical and Neurochemical Aspects of Methamphetamine Dependence

Techniques

Decades of research have provided insight into the neurobiological mechanisms of learning that are thought to be altered following exposure to psychoactive substances by utilizing various *in vitro* and *in vivo* methodologies. For instance, anatomical lesions or inactivation of particular neurotransmitter systems within a specific brain region prior to evaluation of a behavioral response can provide information on the importance of that region for various behaviors and/or neurotransmitter levels in other interconnected brain areas. Retrograde and anterograde labeling of neurons has characterized neuronal pathways from one brain region to another, as well as connections within specific brain structures. In addition, receptor binding techniques can be used to identify and quantify specific receptor subtypes distinct to specific brain regions. Electrically or pharmacologically evoked neurotransmitter release can be assessed in several different assays and provides insights into endogenous neuronal responses. Analysis of neurotransmitter levels from dissected brain tissue or microdialysis samples using high performance liquid chromatography with electrochemical detection (HPLC-EC) has provided useful information about neurotransmitter levels within specific brain regions following pharmacological and behavioral manipulations. Using receptor selective agonists and antagonists in combination with these techniques can reveal detailed information on the contribution of specific neurotransmitter systems or receptor subtypes on the rewarding effects of a psychoactive drug.

In vivo microdialysis has provided a wealth of information about phasic levels of monoamines in the mesocorticolimbic pathways in response to psychoactive drugs. With this technique, rodents are implanted with a guide cannula and stylet that terminates a few millimeters dorsal to the brain region of interest using stereotaxic surgical techniques. Following 5-7 days of recovery, a microdialysis probe, which snaps into the guide cannula, is implanted 4-24 hr prior to an experimental manipulation. The probe has an inlet and outlet tube that allows perfusion of artificial cerebrospinal fluid into and dialysate fluid out of a specified brain region, usually at a rate of ~1 µl/min. At the end of the plastic probe is a 2 mm, semi-permeable membrane across which only small (up to 30 kD) endogenous

molecules can diffuse. Before the experimental manipulation begins, the concentration of molecules inside and outside (i.e. the extracellular space) of the probe reach equilibrium as a result of diffusion down their concentration gradient (Zhang & Beyer, 2006). The dialysate fluid is collected at set time intervals (usually 20 min) following an experimental manipulation and subsequently analyzed using high HPLC-EC. Since baseline samples are collected for each animal, experimental data is generally represented as a percent of baseline and the amount of neurotransmitter represented is derived from external standards. In order to determine the actual concentration of the analyte of interest *in vivo,* a quantitative microdialysis technique known as no-net-flux is often used (Parsons & Justice, 1994). With this method, several concentrations of the analyte of interest are perfused through the microdialysis probe and the dialysate samples are then analyzed. The concentration at which the perfusate and the dialysate are equal is the point of no-net flux, which estimates the concentration of the analyte in the extracellular space (Watson, Venton, & Kennedy, 2006).

By analyzing the microdialysis samples with HPLC-EC, the concentration of catecholamines, in addition to several other endogenous molecules, can be determined. In this assay, the microdialysis sample can be introduced via injection into the mobile phase, which is continuously pumped under pressure through a stationary phase, or column, where the analytes of interest are eluted based on their interactions with these two phases. The mobile phase then carries the analytes to the electrochemical detector where a potential is applied and the analytes are oxidized or reduced and the resulting free electron is recorded as a change in current, which can be further characterized by computer software. Due to the sample volume required for analysis with HPLC-EC, usually ~20 µl, microdialysis with HPLC-EC is useful when assessing an overall increase in monoamine levels across 20 min; however, the temporal resolution does not allow for sec-by-sec analysis of neurochemical changes as can be obtained with voltammetry (Westerink, 1995). However, information provided by voltammetry experiments does not provide the level of sensitivity provided by HPLC-EC (Westerink, 1995). Combinations of the aforementioned techniques in preclinical research designs can provide useful insights into the neurochemical mechanisms underlying the addictive properties of psychostimulants.

Neurocircuitry and Neurotransmitter Systems

Specific anatomical locations in brain have been associated with the primary reinforcing effects of psychostimulants and reward-related learning including: (1) the nucleus accumbens, which is an important region for reward and motor integration; (2) the ventral tegmental area, which is activated by reward and unpredicted events; (3) the prefrontal cortex, which is employed for executive function, impulse control, and decision making; (4) the ventral pallidum, which regulates voluntary motor output; (5) the amygdala, which is essential for emotional processing and reward learning; and (6) the hippocampus, which is critical for memory and detecting novelty (Bardo, 1998). These brain regions communicate with one another through various pathways (Ikemoto, 2007; Kalivas & O'Brien, 2007; Kelley, 2004; Kelley & Berridge, 2002). Dopaminergic cell bodies within the ventral tegmental area project axons to several forebrain regions including the prefrontal cortex, nucleus accumbens, and the basolateral amygdala. The prefrontal cortex in turn sends glutamatergic input back to the ventral tegmental area and to the nucleus accumbens, an area that also receives glutamatergic input from the basolateral amygdala and the hippocampus. This circuitry has been implicated as playing a major role in reward-related learning, decision making, and memory. In addition, glutamate is thought to modulate midbrain dopamine neurons, as stimulation of glutamatergic afferents from the pedunculopontine tegmental nucleus increase burst firing in A9 (substantia nigra) dopaminergic neurons (Lokwan, Overton, Berry, & Clark, 1999). Within the midbrain, GABA afferents from striatonigral neurons cause inhibition of dopamine neuron activity (Grace & Bunney, 1985). Furthermore, when acute methamphetamine (0.15 mg/kg, i.v.) is administered to human subjects, functional magnetic resonance indicates significant activation of the medial orbitofrontal cortex, anterior cingulate cortex (rostral portion), and ventral striatum (Vollm et al., 2004). The interactions of dopaminergic and glutamatergic signaling are critical for reward-related learning and play a role in drug-induced neuronal plasticity, in part due to the co-localization of receptors in medium spiny neurons of the striatum (Berke & Hyman, 2000; Kelley, 2004; Smith & Bolam, 1990). While dopamine signaling appears to be important for detecting unexpected reinforcement, as well as motivation and incentive, the glutamatergic signal is important for sensory/motor processing (Kelley, 2004).

In addition to glutamate, a number of other neurotransmitters are known to modulate mesocorticolimbic dopamine system, including acetylcholine. Acetylcholine is the endogenous neurotransmitter that activates nicotinic and muscarinic receptors. There are two major cholinergic systems in brain, one that arises in the basal forebrain and projects to the cortex and hippocampus and another that arises in the pedunculopontine tegmentum which sends ascending projections to the thalamus and midbrain areas, including the substantia nigra and ventral tegmental area (Dani & De Biasi, 2001). Importantly, activation of the nicotinic receptors that are expressed on presynaptic dopamine neurons can lead to increased neuronal firing in dopaminergic pathways (Dani & De Biasi, 2001).

Dopamine that is released from the presynaptic terminal into the synapse diffuses and interacts with both pre and postsynaptic targets. The dopamine receptors are classified as either D1 or D2-like and are G-protein coupled receptors. D2 receptors function mainly as presynaptic autoreceptors. Stimulation these receptors results in a decreased firing rate and diminished dopamine output from the nerve terminal, while antagonism of the presynaptic D2 receptors results in increased dopamine synthesis and release (Grace, 2002). Activation of D2 receptors results in the inhibition of adenylate cyclase, which subsequently reduces the phosphorylation of cAMP-regulated phosphoprotein of 32,000kDa (DARPP-32; Cooper et al., 2003). When DARPP-32 phosphorylation is reduced, the resulting decrease in protein phosphatase 1inhibition leads to the dephosphorylation of several proteins and ultimately inhibition of neurotransmitter release (Cooper et al., 2003). The D1 receptors are primarily located on postsynaptic dendrites. Activation of these receptors results in an increase in adenylate cyclase, which subsequently increases the phosphorylation of DARPP-32 (Cooper et al., 2003). Increased phosphorylation of DARPP-32 results in potent inhibition of protein phosphatase 1, which interacts with intracellular signaling mechanisms thought to underlie synaptic plasticity.

In addition, extracellular dopamine interacts with the dopamine transporter, which functions normally as a reuptake mechanisms allowing dopamine to be taken back into the presynaptic nerve terminal (Cooper, 2003). Once dopamine is taken back into the presynaptic nerve terminal, it is sequestered in vesicular membranes which protect it from oxidation by monoamine oxidase. The vesicular monoamine

transporter is a protein located in the synaptic vesicular membrane and is responsible for transporting dopamine into the vesicles. Within dopamine neuron terminals, the vesicular monoamine transporter also plays an important role in mediating dopamine release (Schuldiner, 1994).

Methamphetamine is a derivative of amphetamine and has a very similar pharmacology (Melega, Williams, Schmitz, DiStefano, & Cho, 1995). Amphetamines have several molecular targets including: 1) the dopamine transporter; 2) monoamine oxidase activity; and 3) the vesicular monoamine transporter (Mantle, Tipton, & Garrett, 1976; Seiden, Sabol, & Ricaurte, 1993). Amphetamines enter the neuronal cytoplasm by diffusing across the bi-lipid layer and acting as substrates for the dopamine transporter. Once inside, amphetamine inhibits the vesicular monoamine transporter from sequestering dopamine into vesicles and induces the reverse transport of cytosolic dopamine by the dopamine transporter (Ary & Komiskey, 1980; Brown, Hanson, & Fleckenstein, 2000; Liang & Rutledge, 1982; Philippu & Beyer, 1973; Sulzer et al., 1995). In addition to inhibition of the vesicular monoamine transporter, methamphetamine enters the vesicles which results in an altered pH gradient ("weak base" effect) that further decreases the sequestration of neurotransmitter into vesicles (Sulzer et al., 1995). Since amphetamine inhibits monoamine oxidase from metabolizing dopamine, the increased amount of cytosolic dopamine available for amphetamine-induced reverse transport results in an increase in extracellular dopamine and a decrease in the dopamine metabolite DOPAC in the terminal and cell body regions of midbrain dopamine neurons (Dwoskin & Crooks, 2002; Mantle et al., 1976).

A few differences between the neurochemical actions of amphetamine and methamphetamine are emerging. For example, amphetamine has been shown to be more effective than methamphetamine at increasing extracellular dopamine concentrations in the PFC; however amphetamine and methamphetamine exhibit similar efficacy at increasing extracellular dopamine concentrations in the nucleus accumbens (Shoblock, Maisonneuve, & Glick, 2003). In addition, while the norepinephrine transporter functions in concert with the dopamine transporter to remove dopamine from the synapse following its release, amphetamine has a higher binding affinity than methamphetamine for the norepinephrine transporter. As a result, amphetamine is able to block the reuptake of and reversal of both the

norepinephrine and the dopamine transporter, while methamphetamine blocks primarily the dopamine transporter (Shoblock, Maisonneuve, & Glick, 2004). Therefore, by acting through an additional mechanism, amphetamine administration results in a greater concentration of extracellular dopamine in the mPFC than is produced by methamphetamine.

Behavioral Aspects of Drug Dependence

Operant Conditioning

Individuals engage in many behaviors that have specific, reliable outcomes, such as feeling satiated after eating; if the outcome resulting from a particular behavior subsequently leads to an increase of that behavior, that outcome is said to be reinforcing (Koob, 1992). Operant conditioning principles are used in animal models to assess the reinforcing effects of drugs. Typically, animals are prepared with an indwelling jugular catheter and trained to emit a response (i.e. pressing a lever) in order to receive an infusion of a psychoactive drug. Most drugs that are abused by humans are also self-administered by rodents (Gardner, 2000; Koob, 1992). This model allows for the preclinical assessment of interventions that will potentially decrease drug-taking behavior. Behaviors that are reinforced, including those that are biologically relevant, such as eating and engaging in sex, as well as those that lead to the administration of psychoactive drugs, increase dopaminergic neuronal activity in a number of structures and pathways (Kelley, 2004).

Both *d* and *l*-isomers of methamphetamine are self-administered by rats (Pickens, 1967; Yokel & Pickens, 1973). The mesolimbic dopaminergic pathway is critically involved in amphetamine self-administration, as dopamine agonists such as apomorphine and piribedil decrease drug intake, whereas dopamine antagonists such as (+)-butaclamol induce periods of increased drug intake (Yokel & Wise, 1976, 1978). This increase in responding likely reflects attenuation of the rewarding effect of the drug, prompting the animal to increase operant responding to compensate for the decreased drug effect. Lesions of the nucleus accumbens also result in attenuation of amphetamine self-administration, further demonstrating a critical role of this part of the circuitry in drug reward (Lyness, Friedle, & Moore, 1979).

Pavlovian Conditioning

Pavlovian, or classical conditioning, is a form of associative learning that is also important for aspects of drug dependence. In classical conditioning, a temporal contingency is arranged between two stimuli such that one stimulus (the conditioned stimulus) reliably predicts the occurrence of a second stimulus (the unconditioned stimulus; Siegel, 1977). Prior to any pairings with the unconditioned stimulus, the conditioned stimulus typically does not elicit a physiological response. Presentation of the unconditioned stimulus alone will produce a physiological response in an organism, which is known as the unconditioned response. However, following repeated pairings of the conditioned stimulus with the unconditioned stimulus, presentation of the conditioned stimulus alone will come to elicit a physiological response known as the conditioned response. Pavlov (1927) was first to demonstrate that a psychoactive drug is able to function as an unconditioned stimulus. After repeatedly pairing the systemic effects of a psychoactive drug (unconditioned stimulus) with a tone (conditioned stimulus), presentation of the tone alone produced a physiological response similar to the drug action (Pavlov, 1927). These principles can be used to understand the conditioned stimulus-reward associations that are observed following repeated drug administration.

In humans, exposure to environmental stimuli that have become associated with the psychoactive properties of a drug can, by themselves, elicit conditioned responses that often lead to intense craving and relapse (O'Brien, Childress, & McLellan, 1991; Stewart, 1992). When detoxified cocaine users were shown videotapes of simulated cocaine use, they reported cocaine craving (Childress et al., 1999). Concurrent positron emission tomography (PET) assessed in this study indicated that subjects also displayed increased regional cerebral blood flow, an indicator of increased neuronal activity, in limbic regions, including the amygdala and anterior cingulate (Childress et al., 1999). Furthermore, when cocaine-dependent males were read a script of autobiographical drug-related events, neuronal activation was observed in the amygdala, anterior cingulate, and nucleus accumbens (Kilts et al., 2001). More recent studies have revealed that exposure to conditioned stimuli in cocaine and amphetamine-dependent individuals results in increased dopamine release in striatal regions (Boileau et al., 2007; Volkow et al., 2006). These studies illustrate that conditioned cues cause neurophysiological

changes in brain regions important for reward-related learning and the formation of stimulus-reward associations.

Non-Associative Learning

While both operant and Pavlovian conditioning processes are important for understanding drug addiction, non-associative mechanisms also play a role. It has long been recognized that repeated administration of a psychostimulant induces an enduring increase in locomotor activity and at least some of this change is due to non-associative learning. Induction and expression of sensitization in an animal model is thought to reflect changes that occur in the process of human drug addiction (White & Kalivas, 1998). Thus, there has been a focused effort to fully characterize the neurobiological mechanisms involved in learning and memory because these mechanisms are thought to underlie the maladaptive behavioral patterns associated with acquiring, ingesting, and craving psychoactive substances (Kelley, 2004).

Conditioned Place Preference

Conditioned place preference (CPP) is an increasingly utilized paradigm that is used to assess the rewarding properties of psychoactive drugs (for review Bardo & Bevins, 2000; Tzschentke, 1998, 2007). Following repeated administration of a psychoactive drug with a previously neutral environment, the cues associated with that environment will take on secondary rewarding characteristics (i.e. the cues become conditioned). Once this conditioning has occurred, exposure to the conditioned cues results in approach behavior. This paradigm is well characterized for many drugs of abuse and there are insights into the neuroanatomical regions involved in this type of learning that have been derived from microinfusion and lesioning studies (McBride, Murphy, & Ikemoto, 1999; Sellings & Clarke, 2003; Tzschentke, 2007).

There are several variations in protocols used for CPP studies. Typically, a three-compartment apparatus is used in which two larger end compartments are connected with a smaller center compartment. All three compartments are distinct (i.e. varying in color, floor texture, odor, etc.) and guillotine doors allow access to all

three compartments or confinement to one of the end compartments. The three phases that take place during a typical CPP experiment are: (1) preconditioning test, (2) conditioning phase, and (3) postconditioning test. During the preconditioning test, animals are allowed free access to all three compartments and time spent in each is recorded. In an unbiased design, animals show no preference for any of the experimental compartments during the preconditioning test as measured by the time spent in each of the compartments. Animals are then assigned randomly to an experimental group, and which end compartment will serve as the "drug-paired" compartment is counterbalanced across all the animals. In contrast, when animals show a preference for one of the end compartments during the preconditioning test and the opposite end compartment (the non-preferred side) serves as the "drug-paired" compartment during conditioning trials, the design is said to be biased. The biased design is used less often as the interpretation of the results derived from using this design is sometimes problematic (Bardo & Bevins, 2000; Tzschentke, 2007).

Next, during the conditioning phase, animals undergo an experimental manipulation prior to being confined to one of the end compartments during one session and undergo a control session prior to being placed in the opposing end compartment in a separate session. Typically, the experimental and control manipulations are conducted on alternating days. Following completion of the conditioning phase, animals undergo the postconditioning test where they are once again allowed free access to the entire apparatus and time spent in each compartment is assessed. If significantly more time is spent in the compartment paired with the experimental manipulation than that paired with the control condition or than the time spent in that compartment during the preconditioning test, that manipulation induces a *place preference*. If significantly less time is spent in the compartment paired with the experimental manipulation than that paired with the control condition or than time spent in that compartment during the preconditioning test, that manipulation induces a *place aversion*. Two separate aspects of CPP can be assessed, acquisition and expression. During a test of acquisition, animals are conditioned as described above and the animal undergoes the postconditioning test in a drug-free state. Experimental manipulations in this type of design always occur prior to or during the conditioning phase. Alternatively, during a test of expression

animals are conditioned as described above and administered an experimental manipulation (i.e. potential pharmacotherapy), prior to the postconditioning test.

There are several acceptable ways in which to report results from CPP experiments using an unbiased design. The most straightforward method is to compare the time spent (sec) in the previously drug-paired versus saline-paired compartments. Alternatively, a difference score for each group can be calculated by subtracting the time spent (sec) in the previously saline-paired compartment from the time spent in the previously drug-paired compartment. This latter method provides a single number for each experimental group which is advantageous when assessing correlations between CPP data and another dependent variable, such as locomotor activity. Another common approach is to calculate a preference ratio using the following equation: (time spent in drug paired compartment) / (time spent in drug + saline paired compartments). This ratio provides an index of preference for the previously drug-paired compartment.

As with any preclinical model of reward/reinforcement, there are advantages and disadvantages of the CPP paradigm, relative to the drug self-administration paradigm, (Bardo & Bevins, 2000; Van der Kooy, 1987). The advantages of using CPP include: (1) observation of either a preference or an aversion following an experimental manipulation; (2) animals can be assessed for reward-related behavior while in a drug-free state, ensuring that any behavior exhibited is not due to drug-induced impairments; (3) given that animals do not typically have to undergo surgical procedures and the experimental sessions are not as time consuming, these experiments are more cost-efficient; and (4) two behaviors, locomotor activity and reward, can be assessed simultaneously. There are several disadvantages as well, including: (1) difficulty in obtaining a graded dose-effect curve; (2) when using a biased design, it is difficult to determine if the testing apparatus is truly unbiased across studies, making the results ambiguous; and (3) a CPP paradigm for human subjects has not been developed. It appears that the neurocircuitry underlying the classical conditioning processes that occur during CPP are distinct from those underlying the instrumental learning that occurs during drug self-administration (Bardo & Bevins, 2000).

Amphetamine, as well as methamphetamine, has been shown to induce CPP. The acquisition of amphetamine CPP is blocked by both D1 and D2

dopamine receptor antagonists, while only D1 receptor antagonism blocks the expression of amphetamine CPP (Hoffman & Beninger, 1989; Liao, Chang, & Wang, 1998; Spyraki, Fibiger, & Phillips, 1982). Amphetamine infused directly into the nucleus accumbens results in CPP, an effect that is abolished by co-infusion of a D1 antagonist into the nucleus accumbens, as well as by a 6-hydroxydopamine lesion of the nucleus accumbens shell (Carr & White, 1983; Hiroi & White, 1991). In addition to the nucleus accumbens, other brain regions in the limbic circuitry have been implicated in amphetamine-induced CPP. Lesions of the cholinergic pedunculopontine tegmental nucleus block the acquisition of amphetamine CPP, while electrolytic lesions of the lateral nucleus of the amygdala block both acquisition and expression of amphetamine CPP (Olmstead & Franklin, 1994). Interestingly, while medial prefrontal cortex lesions block the acquisition of cocaine CPP, these lesions have no effect on amphetamine CPP (Tzschentke, 1998). Amphetamine CPP is also blocked by reserpine, a VMAT2 ligand that depletes vesicular stores of dopamine (Hiroi & White, 1990). Thus, while acquisition and expression of amphetamine CPP can be affected differentially, dopaminergic neurotransmission in the nucleus accumbens plays a critical role for both.

Locomotor activity during the conditioning phase of CPP experiments is reported frequently and it has been demonstrated that amphetamine-induced locomotor stimulation is not necessary for its rewarding properties (Carr, Phillips, & Fibiger, 1988). Amphetamine sensitization has been shown to be, at least in part, dependent on contextual cues because the sensitized response is not observed if d-amphetamine is administered in a novel environment (Anagnostaras & Robinson, 1996). Many neurotransmitter systems have been implicated in the process of sensitization, including dopamine, glutamate, and acetylcholine (White & Kalivas, 1998).

To what extent the outcomes of CPP, self-administration, and locomotor activity experiments assessing psychostimulant-induced behaviors are correlated is currently under debate. Sensitization is thought to occur during the acquisition of psychostimulant self-administration (Schenk & Partridge, 1997). While amphetamine-induced behaviors rely on the mesocorticolimbic dopamine pathway and often occur simultaneously, they are dissociable.

Lobeline as a Potential Pharmacotherapy for Methamphetamine Dependence

Recently, it has been postulated that lobeline, an active alkaloid found in Indian tobacco (*Lobelia inflata*), may have potential as a pharmacotherapy for psychostimulant abuse (Dwoskin & Crooks, 2002). Lobeline has a complex pharmacological profile. This alkaloid has been classified historically as a nicotinic receptor agonist that binds more selectively to high than to low affinity nicotinic receptors in striatal preparations (Brioni, Decker, Sullivan, & Arneric, 1997; Decker, Majchrzak, & Arneric, 1993). However, several lines of evidence now indicate that it acts as a functional nicotinic receptor antagonist. *In vitro* studies have shown that lobeline inhibits [^{3}H]overflow from superfused [^{3}H]dopamine-preloaded striatal slices and acts as a functional antagonist as assessed in the rubidium efflux assay using thalamic synaptosomes (D. K. Miller, Crooks, & Dwoskin, 2000; Teng, Crooks, Sonsalla, & Dwoskin, 1997). *In vivo* microdialysis experiments in nicotine pretreated rats (0.4 mg/kg, SC for 5 days) have demonstrated that systemic lobeline administration does not change extracellular levels of dopamine or 3, 4-dihydroxyphenylacetic acid (DOPAC) in the nucleus accumbens core (Benwell & Balfour, 1998). Furthermore, repeated administration of nicotine results in increased nicotinic receptor binding sites, whereas repeated administration of lobeline does not alter the affinity or number of nicotinic receptor binding sites (Bhat, Turner, Selvaag, Marks, & Collins, 1991). Consistent with the neurochemical evidence that lobeline acts as a functional nicotinic receptor antagonist; administration of lobeline specifically attenuates the locomotor-stimulating effects of repeated nicotine administration (Miller et al., 2003). Thus, evidence from both neurochemical and behavioral research strongly supports the view that lobeline is a functional antagonist at neuronal nicotinic receptors in the mesolimbic dopamine system.

In addition to the aforementioned activity at neuronal nicotinic receptors, lobeline also interacts with the vesicular monoamine transporter to inhibit the sequestration of dopamine into vesicular stores (Teng, Crooks, & Dwoskin, 1998). It is thought that through this mechanism, lobeline has potential as a pharmacotherapy for methamphetamine dependence (for review see (Dwoskin & Crooks, 2002). Lobeline pretreatment selectively inhibits amphetamine induced dopamine overflow in rat striatal slices as assessed in the endogenous dopamine

release assay (Miller et al., 2001). These *in vitro* experiments have indicated that in the presence of lobeline, presynaptic dopamine stores are redistributed, rendering dopamine unavailable for methamphetamine-induced reverse transport (Dwoskin & Crooks, 2002). It is likely that lobeline attenuates the rewarding properties of methamphetamine through VMAT2 inhibition, although other mechanisms may also play a role.

Lobeline may have potential as a pharmacotherapy for treating methamphetamine dependence (Dwoskin & Crooks, 2002; Zheng, Dwoskin, & Crooks, 2006). Using a rodent model of methamphetamine self-administration, the potential of lobeline to decrease drug taking behavior was assessed (Harrod, Dwoskin, Crooks, Klebaur, & Bardo, 2001). Male Sprague-Dawley rats were trained to lever press for sucrose reinforcement, underwent surgery to implant an indwelling jugular catheter, and were then trained to self-administer methamphetamine (0.05 mg/kg/infusion) on a terminal fixed ratio 5 schedule of reinforcement. A separate group of rats was trained to lever press for sucrose reinforcement on a terminal fixed ratio 5 schedule of reinforcement. Once stable responding was reached in both assays, lobeline (0.3, 1.0 and 3.0 mg/kg, sc) pretreatments were administered. The highest dose of lobeline (3 mg/kg) resulted in an acute decrease of both methamphetamine infusions and sucrose pellets. Additional experiments demonstrated that following 7 repeated administrations, lobeline (3 mg/kg) selectively decreased methamphetamine infusions as tolerance developed to the decrease in sucrose pellets earned (Harrod et al., 2001). Further, increasing the unit dose of methamphetamine does not surmount the lobeline-induced decrease (Harrod et al., 2001). While acute lobeline (3 mg/kg) administration prior to methamphetamine (1.0 mg/kg) did not specifically decrease methamphetamine-induced reinstatement of methamphetamine-seeking behavior, an overall decrease in number of responses was observed (Harrod, Dwoskin, Green, Gehrke, & Bardo, 2003). However, given that lobeline (3 mg/kg) has been shown to non-specifically decrease operant behavior acutely, an effect that tolerates within four administrations, it is unknown if repeated lobeline administration would result in specific attenuation of methamphetamine-induced reinstatement of methamphetamine-seeking behavior. It is also currently unknown if lobeline would decrease cue-induced reinstatement of methamphetamine-seeking behavior. While

there are currently no reports on human studies assessing lobeline's efficacy as a pharmacotherapy for methamphetamine dependence in a clinical population, research from preclinical models appear promising.

Importantly, lobeline does not appear to have abuse liability. In one study, four separate groups of male Sprague-Dawley rats were trained to lever press for sucrose reinforcement, implanted with a jugular catheter, and allowed to self-administer either saline or one dose of lobeline (0.015, 0.05, 0.15 mg/kg/infusion; (Harrod et al., 2003). None of the rats acquired self-administration of lobeline at any of the doses tested. In addition, acute lobeline (1 and 3 mg/kg) does not reinstate extinguished methamphetamine-seeking behavior (Harrod et al., 2003). Further, lobeline administration repeatedly paired with a distinct environment does not induce a conditioned place preference (Fudala & Iwamoto, 1986). These studies illustrate the utility of lobeline to inhibit reward-related behaviors induced by psychostimulants without functioning as a reinforcer when administered alone.

As indicated previously, pharmacological manipulations can differentially affect the acquisition and expression of CPP. While the effects of lobeline on acquisition of methamphetamine CPP can provide insight into potential mechanisms underlying the ability of lobeline to decrease methamphetamine reward, it is also clinically relevant to determine if lobeline attenuates the expression of methamphetamine CPP because a pharmacotherapy would be administered following previous exposure to a psychoactive substance. Previous reports indicate that administration of lobeline alone does not elicit drug-seeking behavior in rats extinguished from methamphetamine-taking behavior (Harrod et al., 2003). However, there is currently no available information of the effect of lobeline on cue-induced reinstatement of drug-seeking behavior.

Since methamphetamine CPP can be disrupted by a variety of neuroanatomical and pharmacological manipulations, it is also of interest to assess the neurochemical profile of lobeline administration prior to methamphetamine *in vivo*. Currently, the proposed mechanisms by which lobeline is decreasing methamphetamine reward are derived mostly from *in vitro* experiments. Given the complexity of the limbic system pathways that are disrupted during tissue extraction, it is essential that these effects are confirmed by *in vivo* experiments.

CHAPTER TWO

Experiment 1

Lobeline Attenuates the Acquisition of Methamphetamine-Induced Conditioned Place Preference and Locomotor Activity.

The purpose of the first experiment was to assess if lobeline alters methamphetamine CPP in rats. In contrast to self-administration, CPP is a model that assesses the role of conditioned contextual cues in drug reward. In humans, exposure to environmental or contextual cues that are associated with drugs of drug through classical conditioning can elicit drug craving and relapse (O'Brien et al., 1991). When tested in a drug-free state, re-exposure to amphetamine-associated cues in healthy human volunteers increases dopaminergic transmission in the ventral striatum similar to the increase observed following amphetamine administration (IV) (Boileau et al., 2007; Drevets et al., 2001). Since lobeline has been proposed to be a potential pharmacotherapy for methamphetamine dependence, the ability of lobeline to disrupt the conditioned associations that are formed by repeated pairings of methamphetamine with distinct contextual cues warrants examination. In addition to assessing the effects of lobeline on methamphetamine CPP, the current preclinical study also measured locomotor activity during drug-conditioning trials. It was hypothesized that lobeline would attenuate methamphetamine-induced hyperactivity and inhibit the acquisition of methamphetamine-induced CPP.

Methods

Subjects

Male Sprague-Dawley rats (N=110, 225-250 g; Harlan Industries, Indianapolis, IN) were housed two per cage, with *ad libitum* access to food and water. The colony room was maintained on a 12-hr/12-hr light/dark cycle and controlled for temperature and humidity. All animals were handled at least 5 min on each of the 3 days prior to commencement of the experiment. Experiments were conducted during the light phase. All experimental protocols were conducted in strict accordance with the National Institutes of Health Guide for the Care and Use

of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

Drugs

Methamphetamine hydrochloride was obtained from the National Institute on Drug Abuse (Research Triangle Park, NC, USA). Lobeline hemisulfate was purchased from ICN (Costa Mesa, CA). Doses of methamphetamine and lobeline were calculated as salt weight, were dissolved in 0.9% NaCl (saline) and were administered in 1 ml/kg volume. Lobeline was administered via SC injection and methamphetamine was administered via IP injection.

Apparatus

CPP and locomotor activity were assessed using an automated, 3-compartment apparatus operated via a computer interface equipped with MED-PC IV software (ENV-013; Med Associates, St. Albans, VT). The apparatus was 68 x 21 x 21 cm and consisted of three distinct compartments: two 28-cm long side compartments (one colored black with a stainless steel rod floor and one colored white with a stainless steel mesh floor) separated by a 12-cm long central gray compartment with a smooth PVC floor. Guillotine doors separated each side chamber from the central chamber and were manipulated in order to confine rats to one of the side compartments or to allow free access to all three compartments. Inside each side compartment, six photobeams were located 1.25 cm from the end wall and 5 cm apart. Inside the central gray compartment, there were three photobeams spaced 4.75 cm apart.

Experimental Procedure

Twelve separate groups of animals (n=8-11) were used in the current study, making up a 3 x 4 (lobeline dose x methamphetamine dose) experimental design. On Day 1 (preconditioning test), animals were placed individually in the apparatus with both guillotine doors open to allow access to the entire apparatus for 15 min to determine the initial preference. On Days 2-9 (conditioning sessions), animals were confined to each side of the apparatus on alternating days. On drug conditioning days, animals were pretreated with saline or lobeline (1 or 3 mg/kg, SC) and were

placed back into their home cage for 15 min. Animals were then injected with saline or methamphetamine (0.5, 1 or 3 mg/kg, IP) and placed immediately in one of the side compartments (white or black; counterbalanced within treatment group regardless of initial preference) for 30 min. Locomotor activity was recorded as the number of beam breaks on the last drug conditioning trial. On the alternating days, animals were treated similarly except that both injections were saline and the animals were placed into the opposite side compartment. On Day 10 (postconditioning test), preference was assessed by placing the animal in the center grey compartment with free access to all compartments for 15 min. The amount of time spent in each compartment was recorded and CPP was defined as a significant increase in time spent in the drug-paired compartment relative to the saline-paired compartment.

Data Analysis

Locomotor activity was assessed by analyzing the number of horizontal beam breaks during the last drug-conditioning session using a one-way ANOVA across treatment groups. Posthoc analyses were conducted using unpaired t-tests (one-tailed) with correction for family-wise error to determine significant between group differences. Significance level for all analyses was set at $p<0.05$. Preference data were analyzed as time (sec) spent in the saline versus drug-paired compartments during the postconditioning test session using a 12 x 2 (treatment groups x compartment) mixed factor analysis of variance (ANOVA), with treatment groups as a between-subject factor and compartment as a within-subject, repeated measure factor. Posthoc analyses were conducted using paired t-tests (one-tailed) with correction for family-wise error to assess differences in time spent in saline versus drug-paired compartments within each group.

Results

Locomotor Activity

The overall analysis of locomotor activity during the last drug conditioning session revealed a significant main effect of treatment group ($F(11,98)=3.185$; $p<0.001$). Figure 2.1 illustrates that all methamphetamine (0.5, 1.5 and 3 mg/kg) conditioning groups showed a significant increase in locomotor activity compared to

saline control (panel A), while no difference was observed between the saline control and either lobeline (1 and 3 mg/kg) alone groups (panel B).

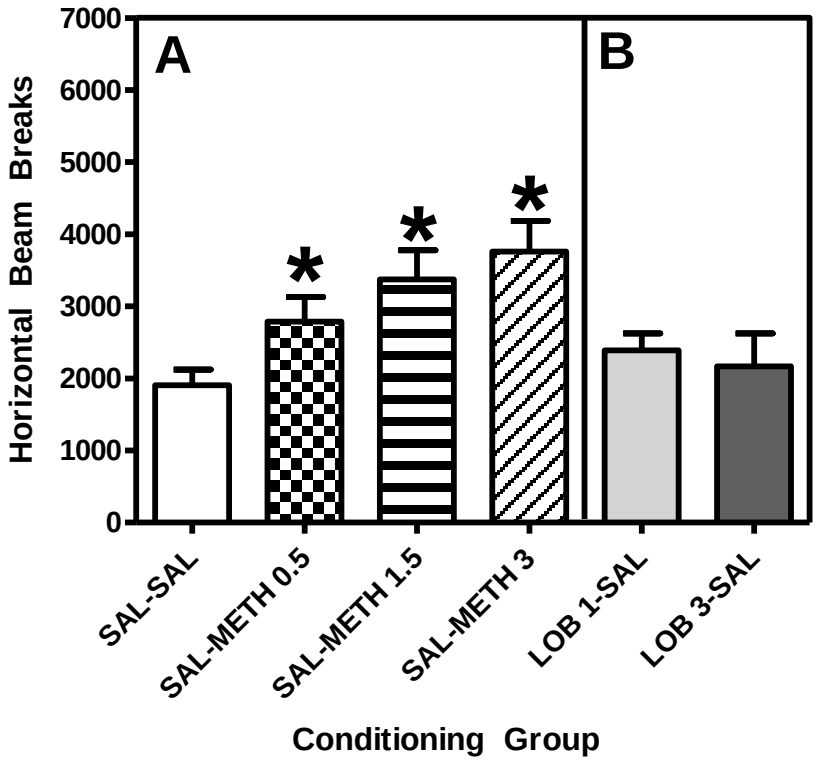


Figure 2.1 Locomotor Activity During the 4th Drug Conditioning Session for Control Groups. Mean (± SEM) horizontal beam breaks in the drug-paired compartment during the last drug conditioning session (Trial 4) following methamphetamine (0, 0.5, 1 or 3 mg/kg) alone (**Panel A**) or lobeline (0, 1 or 3 mg/kg) alone (**Panel B**). Asterisk (*) indicates a significant difference from SAL-SAL group; $p<0.05$.

Figure 2.2 illustrates that lobeline (1 and 3 mg/kg) pretreatment did not alter locomotor activity following the lower methamphetamine doses (0.5 mg/kg; panel A or 1.5 mg/kg; panel B) on the last conditioning session. In addition, the lower dose of lobeline (1 mg/kg) did not significantly alter the effect of 3 mg/kg of methamphetamine (Figure 2.2, panel C). However, 3 mg/kg of lobeline significantly attenuated locomotor activity following the highest dose of methamphetamine (3 mg/kg).

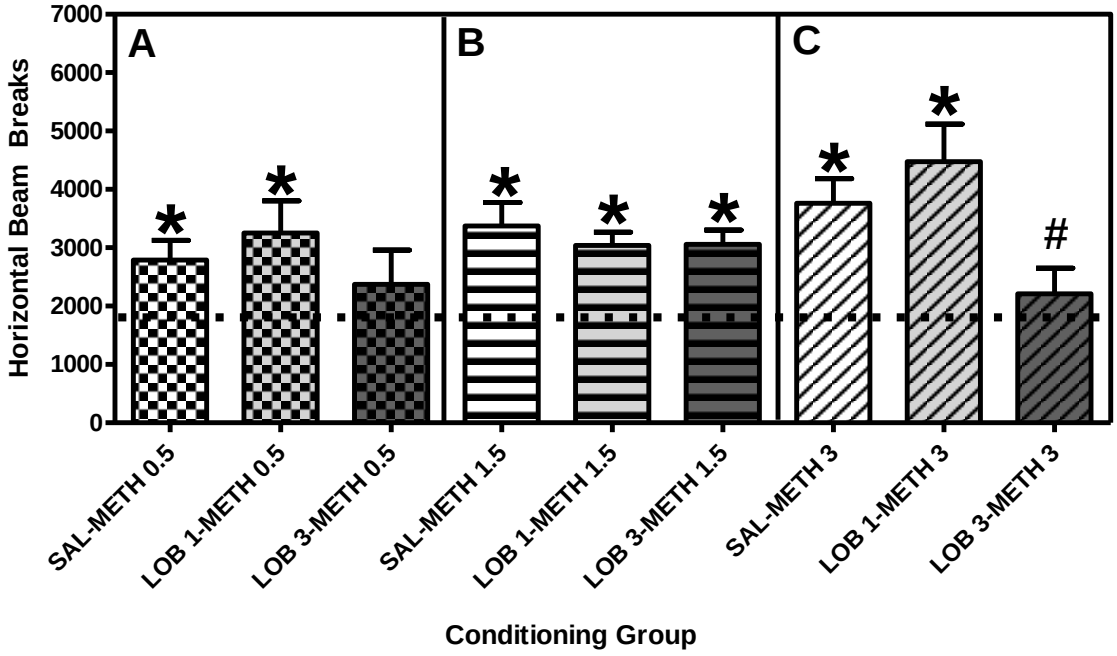


Figure 2.2 Locomotor Activity During the 4th Drug Conditioning Session For Lobeline-Methamphetamine Groups. Mean (± SEM) beam breaks in the drug-paired compartment during the last drug conditioning session (Trial 4) following pretreatment with lobeline (0, 1 or 3 mg/kg) prior to methamphetamine (0, 0.5, 1 or 3 mg/kg). The dashed line represents the number of beam breaks in the SAL-SAL group. **Panel A**: Lobeline (0, 1 or 3 mg/kg) pretreatment combined with methamphetamine (0.5 mg/kg). **Panel B:** Lobeline (0, 1 or 3 mg/kg) pretreatment combined with methamphetamine (1.5 mg/kg). **Panel C:** Lobeline (0, 1 or 3 mg/kg) pretreatment combine with methamphetamine (3 mg/kg). Asterisk (*) indicates a significant difference from SAL-SAL group, which is represented by the dashed line; $p<0.05$. Hatch (#) indicates a significant difference from SAL-METH 3; $p<0.05$.

Acquisition of Conditioned Place Preference

The overall analysis of time spent in each compartment during the postconditioning test revealed significant main effects of treatment group ($F(11,98)=8.06$; $p<0.01$) and compartment (saline or drug-paired; $F(1,98)=70.92$; $p<0.01$). In addition, ANOVA indicated that there was a significant interaction of compartment (saline or drug-paired) x treatment group ($F(11,98)=3.71$; $p<0.01$). Significant CPP was observed with each methamphetamine conditioning dose (0.5,

1.5 and 3 mg/kg; Fig 2.3A), while lobeline alone did not alter preference significantly (Fig 2.3B). In addition, control rats conditioned with saline alone did not show a preference for either compartment during the postconditioning test.

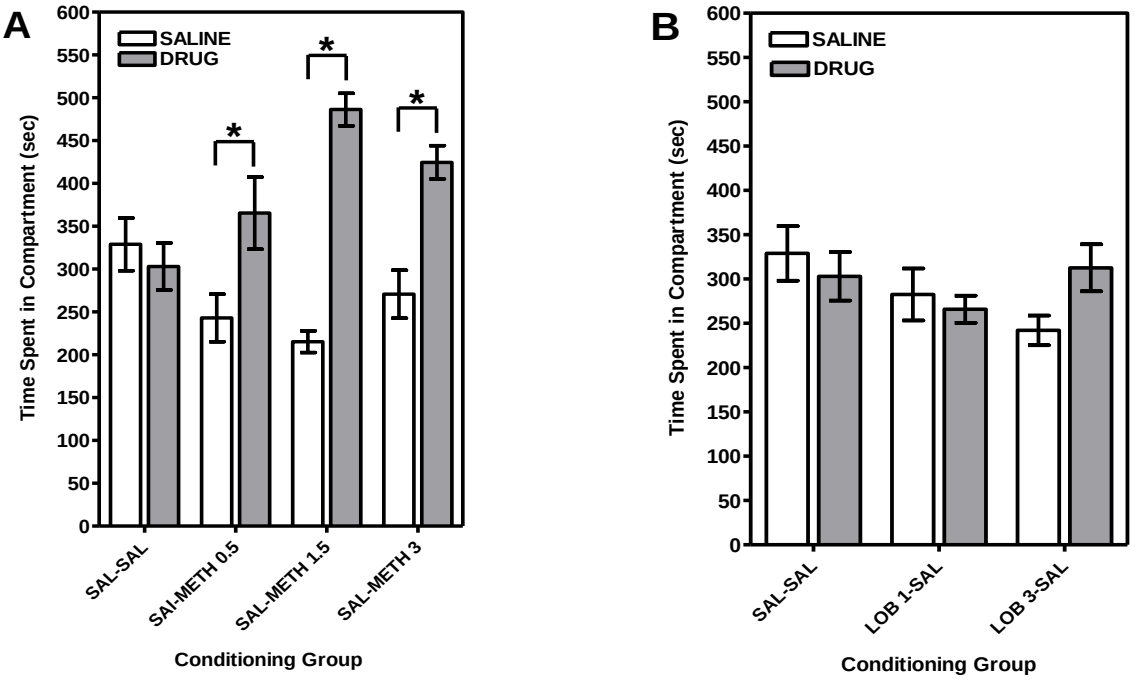


Figure 2.3 Acquisition of CPP for Control Groups. Mean (± SEM) amount of time rats spent in the saline- and drug-paired compartments during the 15 min postconditioning test following methamphetamine (0, 0.5, 1 or 3 mg/kg; **Panel A**) or lobeline (0, 1 or 3 mg/kg; **Panel B**) alone. Asterisk (*) indicates a significant within-subject difference in time spent in the saline versus drug paired compartment; $p<0.05$.

During conditioning with the lowest dose of methamphetamine (0.5 mg/kg), pretreatment with either dose of lobeline (1 or 3 mg/kg) blocked the acquisition of methamphetamine-induced CPP (Figure 2.4 A). However, during conditioning with the higher methamphetamine doses (1.5 or 3 mg/kg), pretreatment with either dose of lobeline (1 or 3 mg/kg) during conditioning had no significant effect on the acquisition of methamphetamine-induced CPP (Figure 2.4B and 2.4C), as rats spent more time in the previously drug-paired compartment during the postconditioning test.

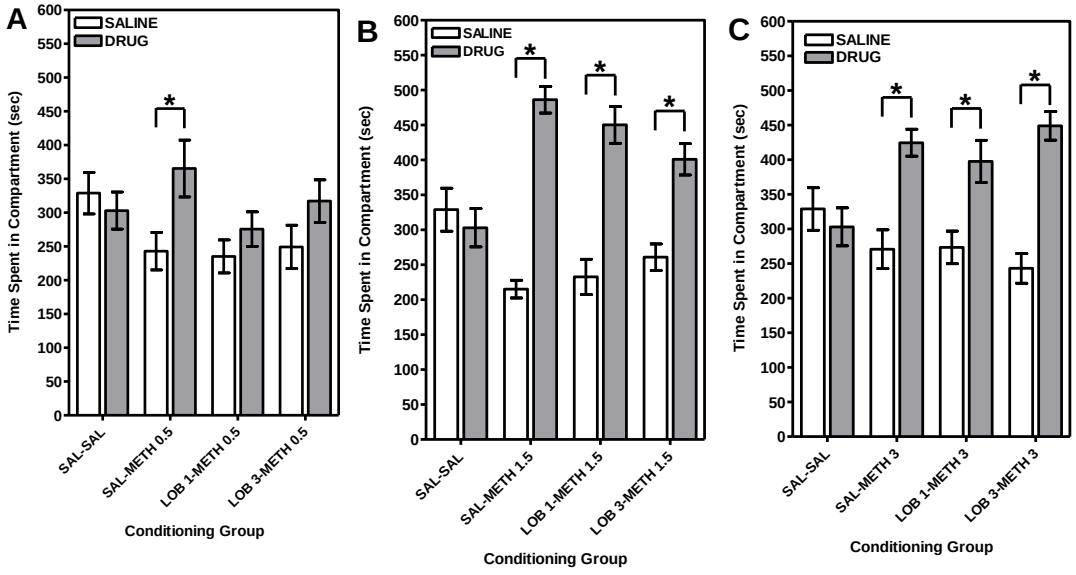


Figure 2.4 Acquisition of CPP in Lobeline-Methamphetamine Groups. Mean (± SEM) amount of time spent in the saline- and drug-paired compartments during the 15-min postconditioning test for the groups pretreated with lobeline (0, 1 or 3 mg/kg) prior to methamphetamine (0, 0.5, 1 or 3 mg/kg) during the conditioning phase. **Panel A**: lobeline (0, 1 or 3 mg/kg) pretreatment combined with methamphetamine (0.5 mg/kg). **Panel B:** Lobeline (0, 1 or 3 mg/kg) pretreatment combined with methamphetamine (1.5 mg/kg). **Panel C:** Lobeline (0, 1 or 3 mg/kg) pretreatment combined with methamphetamine (3 mg/kg). Asterisk (*) indicates a significant within-subject difference in time spent in the saline versus drug paired compartment; $p<0.05$.

Summary of Experiment 1

The aim of the current experiment was to examine the effect of lobeline on methamphetamine-induce locomotor hyperactivity and acquisition of CPP. During the last drug conditioning session, rats were confined to one end compartment and horizontal beam breaks were recorded for the 30 min session. Methamphetamine (0.5, 1.5 and 3 mg/kg) dose dependently induced hyperactivity during the last conditioning session compared to the saline control group, while lobeline (1 or 3 mg/kg) alone did not alter locomotor activity during this session. Previous research suggests that acute lobeline (3 mg/kg) induces non-specific hypoactivity in rats (Harrod et al., 2001; Miller et al., 2001). Lobeline (3 mg/kg) did significantly

attenuate locomotor activity during the first conditioning session in the current study (data not shown). However, tolerance to the hypoactivity was evident within four administrations, as no hypoactivity was evident on the last conditioning session. The lower dose of lobeline (1.0 mg/kg) did not block methamphetamine (0.5, 1.5 or 3.0)-induced hyperactivity. Interestingly, the higher dose of lobeline (3.0 mg/kg) attenuated locomotor hyperactivity only in combination with the highest methamphetamine (3.0 mg/kg) dose administered. When the methamphetamine dose is increased from 1.5 to 3 mg/kg, additional pharmacological consequences may be induced that contribute to the increase in methamphetamine-induced hyperactivity. Lobeline may be interfering with these additional pharmacological mechanisms, resulting in a decrease in methamphetamine-induced locomotor activity.

All rats receiving methamphetamine during the conditioning phase acquired CPP. Importantly, neither dose of lobeline (1 or 3 mg/kg) induced CPP or place aversion, which is congruent with previous findings (Fudala & Iwamoto, 1986). As hypothesized, lobeline (1 and 3 mg/kg) administration prior to methamphetamine (0.5 mg/kg) during the conditioning phase blocked the acquisition of CPP. However, the lobeline-induced blockade was surmounted when rats were conditioned with higher doses of methamphetamine (1.5 and 3.0 mg/kg). Interestingly, previous research from our laboratory has indicated that the ability of lobeline to decrease methamphetamine self-administration is not surmounted by increasing the unit dose of methamphetamine (Harrod et al., 2001). This discrepancy in results obtained from self-administration and CPP paradigms is not unique as previous research has demonstrated that pharmacological manipulations can differentially affect these paradigms (Bardo & Bevins, 2000). The current results demonstrate that lobeline (1 and 3 mg/kg) can decrease the acquisition of CPP induced by a low dose of methamphetamine (0.5 mg/kg). In addition, the lobeline (3 mg/kg)-induced attenuation of methamphetamine (3 mg/kg)-induced hyperactivity was not correlated with a decrease in the rewarding properties of methamphetamine, demonstrating that these two behavioral effects are dissociable.

CHAPTER THREE

Experiment 2

Lobeline Attenuates the Expression of Methamphetamine-Induced Conditioned Place Preference

In order to determine if lobeline attenuates the expression of an established methamphetamine CPP, lobeline was administered prior to postconditioning tests. As discussed in the introduction, the neurochemical mechanisms involved in the acquisition of methamphetamine CPP are dissociable from those involved in the expression of methamphetamine CPP. This is clinically relevant because methamphetamine-induced cellular adaptations and learned associations are generally formed prior to an individual seeking treatment for dependence. The expression of CPP in rodents is thought to model context-conditioned reward in humans. Although no parallel experimental paradigm has been established in humans, it is well recognized that exposure to contextual cues that have been associated previously with drug effects often elicit drug craving. It has been previously reported that lobeline does not specifically attenuate methamphetamine-induced reinstatement of lever responding in rats following extinction of methamphetamine self-administration (Harrod et al., 2003). As such, it was hypothesized that lobeline would not specifically inhibit the expression of methamphetamine-induced CPP.

Methods

Subjects

One hundred and four male Sprague-Dawley rats (n=10-12/group; 225-250 g; Harlan Industries, Indianapolis, IN) were used in the current study. These rats were treated identically to those described in experiment 1.

Drugs

Same as described in Experiment 1.

Apparatus

Same as described in Experiment 1.

Experimental Procedure

Nine separate groups of animals were assigned randomly to one of nine different treatment groups, making up a 3 x 3 factorial design (Table 1).

Table 3.1 Experimental groups for Experiment 2.

Drug Conditioning	Postconditioning Test	n
SAL	SAL	10
SAL	LOB 1 mg/kg	10
SAL	LOB 3 mg/kg	12
METH 0.5 mg/kg	SAL	12
METH 0.5 mg/kg	LOB 1 mg/kg	12
METH 0.5 mg/kg	LOB 3 mg/kg	12
METH 1.5 mg/kg	SAL	12
METH 1.5 mg/kg	LOB 1 mg/kg	12
METH 1.5 mg/kg	LOB 3 mg/kg	12

On Day 1 (preconditioning test), animals were placed in the apparatus with both guillotine doors open to allow access to the entire apparatus during which their initial place preference during a 15-min session was determined. On Days 2-9 (conditioning phase), animals were confined to one of the end compartments and underwent saline or drug conditioning trials on alternating days. On drug conditioning trials, animals were administered saline or methamphetamine (0.5 or 1.5 mg/kg, IP) and immediately placed in one of the end compartments in a counterbalanced manner (white or black; drug paired compartment) for 30 min. These doses of methamphetamine were chosen based on previously published results and based on preliminary experiments from our laboratory indicating that 0.5 mg/kg of methamphetamine is the minimal dose that will result in reliable conditioned place preference (Kuo et al., 2007). On alternate days, animals were injected with saline (IP) and placed in the opposite end chamber (saline paired compartment). On Days 10-11 (postconditioning tests), saline or lobeline (1 or 3 mg/kg, SC) was administered in a counter balanced manner and 15 min later methamphetamine-induced conditioned place preference was assessed by placing the animal in the grey (center) compartment with free access to all compartments

for 15 min. These postconditioning tests are referred to as the 1st saline and 1st lobeline post-tests. On Days 12-15, each rat was administered saline or their respective dose of lobeline for 4 consecutive days and tested in an identical procedure described for the previous postconditioning test in order to assess the effects of repeated lobeline. Days 12-15 are referred to as the 2nd to 5th lobeline post-tests. On Day 16, all animals received saline prior to a postconditioning test in order to assess the persistence of the methamphetamine-induced CPP. Day 16 is referred to as the final saline post-test.

Data Analysis

All data are expressed as group means ± SEM. Initially, horizontal beam breaks in each of the compartments were recorded during each postconditioning test and a rate of locomotor activity was calculated using the following equation: horizontal beam breaks / time spent in compartment. These data were analyzed using a mixed factor ANOVA with group as a between-subject variable and compartment and postconditioning test day as within-subject variables. This analysis indicated no differences in the rate of locomotor activity between the two end compartments so the beam breaks were collapsed in all subsequent analyses. Locomotor activity data are expressed as the total number of horizontal beam breaks in all 3 compartments during each of the postconditioning tests as and were analyzed using a mixed factor ANOVA, with group as a between-subject variable and postconditioning test day as a within-subject variable. Post-hoc analyses were conducted using paired and unpaired t-tests (one-tailed) with correction for family-wise error to determine significant group differences. CPP data were analyzed as time (sec) spent in the saline versus methamphetamine-paired compartments during the postconditioning-test using an overall 3-way mixed factor ANOVA, with group as a between-subject variable and compartment and postconditioning test day as within-subject variables. Subsequent 3-way ANOVAs were conducted for each conditioning group. Post-hoc analyses were conducted using paired t-tests (one-tailed) with correction for family-wise error to determine significant decreases in time spent in the saline versus drug-paired compartments within each conditioning group. In order to assess whether the non-specific hypoactivity typically induced by lobeline confounds the expression of CPP, correlational

analyses were conducted. For these analyses, a difference score was calculated for CPP using the following equation: time spent in drug – time spent in saline. Separate Pearson's correlations were conducted using the CPP difference score and total horizontal beam breaks on each of the postconditioning test sessions. Significance level (alpha) for all analyses was set at $p<0.05$.

Results

Locomotor Activity During Postconditioning Tests

The overall 2-way ANOVA of locomotor activity across postconditioning tests indicated significant main effects of treatment group ($F(8,92)=3.69$; $p<0.01$) and postconditioning test ($F(3,24)=37.18$; $p<0.01$). An interaction of treatment group x postconditioning test was also revealed ($F(24,276)=8.07$; $p<0.01$). Three separate groups of animals were administered saline during the conditioning phase of the experiment and underwent repeated postconditioning test sessions (Figure 3.1). Within the group that was repeatedly challenged with saline during these postconditioning tests, a decrease in activity was observed by the 5th repeated postconditioning test ($p<0.05$). Interestingly, no decrease was observed in rats repeatedly challenged with the lower dose of lobeline (1 mg/kg), indicating an effect indicative of habituation. Administration of the higher dose of lobeline (3 mg/kg) resulted in acute and persistent suppression of locomotor activity ($p<0.05$). However, some tolerance to the suppressant effect was observed between the 1st and 5th lobeline administration ($p<0.05$). No significant decrease was observed between the 1st and final saline challenge sessions, indicating no enduring suppressant effect of lobeline.

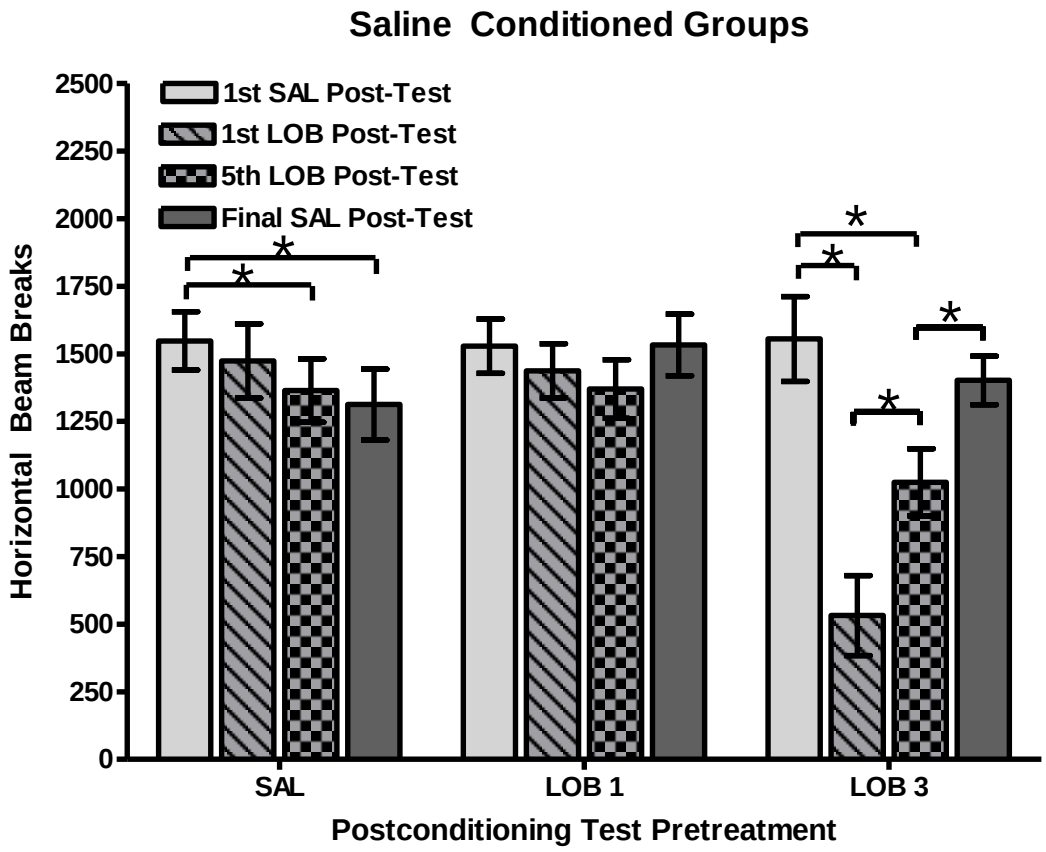


Figure 3.1 Locomotor Activity During Postconditioning Tests in Rats that Received Saline During the Conditioning Phase. Mean (± SEM) horizontal beam breaks in the CPP apparatus following lobeline (0, 1 or 3 mg/kg) pretreatment during the 15-min postconditioning test session. All rats received saline during the conditioning phase. Asterisk **(*)** indicates a significant difference between bars ($p<0.05$).

Three separate groups of animals were administered a low dose of methamphetamine (0.5 mg/kg) during the conditioning phase of the experiment and underwent repeated postconditioning test sessions (Figure 3.2). Rats repeatedly challenged with saline showed no differences across postconditioning test sessions ($p<0.05$). However, a significant decrease was observed following repeated testing with the lower dose of lobeline (1 mg/kg). The higher dose of lobeline (3 mg/kg) resulted in acute and persistent suppression of locomotor activity ($p<0.05$). However, some tolerance to this suppressant effect was observed between the 1st

and 5th lobeline administration ($p<0.05$). A significant decrease was also observed between the 1st and final saline challenge sessions.

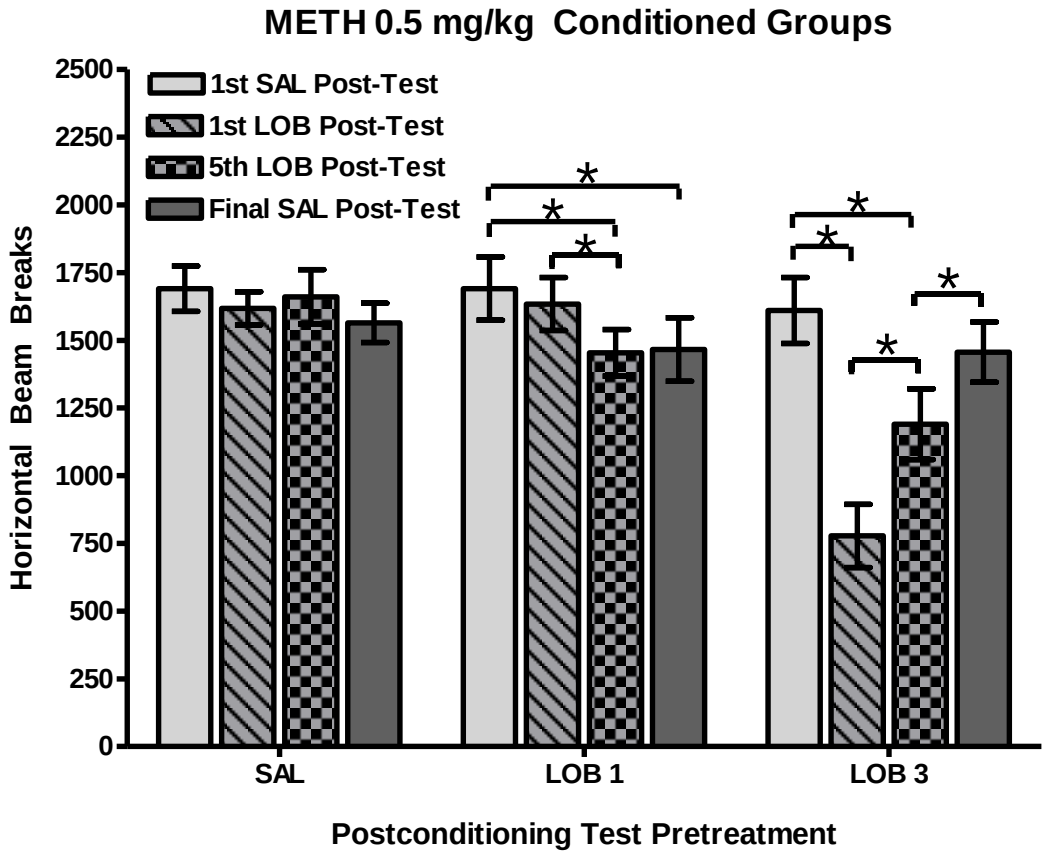


Figure 3.2 Locomotor Activity During Postconditioning Tests in Rats that Received Methamphetamine (0.5 mg/kg) During the Conditioning Phase. Mean (± SEM) horizontal beam breaks in the CPP apparatus following lobeline (0, 1 or 3 mg/kg) pretreatment during the 15-min postconditioning test session. All rats received methamphetamine (0.5 mg/kg) during the conditioning phase. Asterisk (*) indicates a significant difference between bars ($p<0.05$).

Three separate groups of animals were administered a high dose of methamphetamine (1.5 mg/kg) during the conditioning phase of the experiment and underwent repeated postconditioning test sessions (Figure 3.3). Rats repeatedly challenged with saline across postconditioning tests showed a significant decrease by the final saline postconditioning test, suggestive of habituation to the test

chamber (Figure 3.6; $p<0.05$). A decrease was also observed between the 1st saline and last saline postconditioning test sessions in rats repeatedly challenged with the lower dose of lobeline (1 mg/kg). Administration of the higher dose of lobeline (3 mg/kg) resulted in acute and persistent suppression of locomotor activity ($p<0.05$). However, some tolerance to this suppressant effect was observed between the 1st and 5th lobeline administration ($p<0.05$). A significant decrease was also observed between the 1st and final saline challenge sessions.

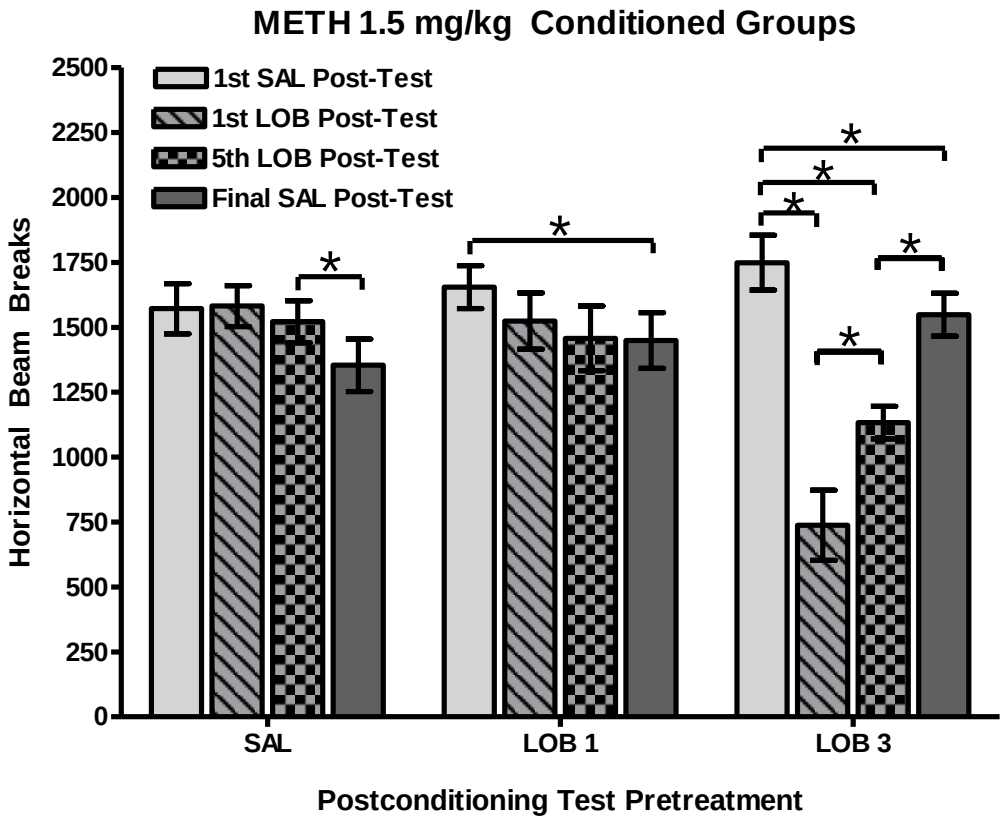


Figure 3.3 Locomotor Activity During Postconditioning Tests in Rats that Received Methamphetamine (1.5 mg/kg) During the Conditioning Phase. Mean (± SEM) horizontal beam breaks in the CPP apparatus following lobeline (0, 1 or 3 mg/kg) pretreatment during the 15-min postconditioning test session. All rats received methamphetamine (1.5 mg/kg) during the conditioning phase. Asterisk (*) indicates a significant difference between bars ($p<0.05$).

Expression of Conditioned Place Preference

The overall analysis of time spent in the saline versus drug paired compartments across 7 consecutive postconditioning test days indicated significant main effects of test day ($F(3,276)=3.07$; $p<0.05$) and compartment ($F(1,92)=25.32$; $p<0.01$). In addition, a significant compartment x group interaction ($F(8,276)=2.01$; $p<0.05$) was revealed. Separate 3-way ANOVAs were conducted for each of the 3 conditioning groups (0, 0.5 and 1.5 mg/kg). There were no significant differences found in the saline conditioned groups (Figure 3.4 Panels A-D).

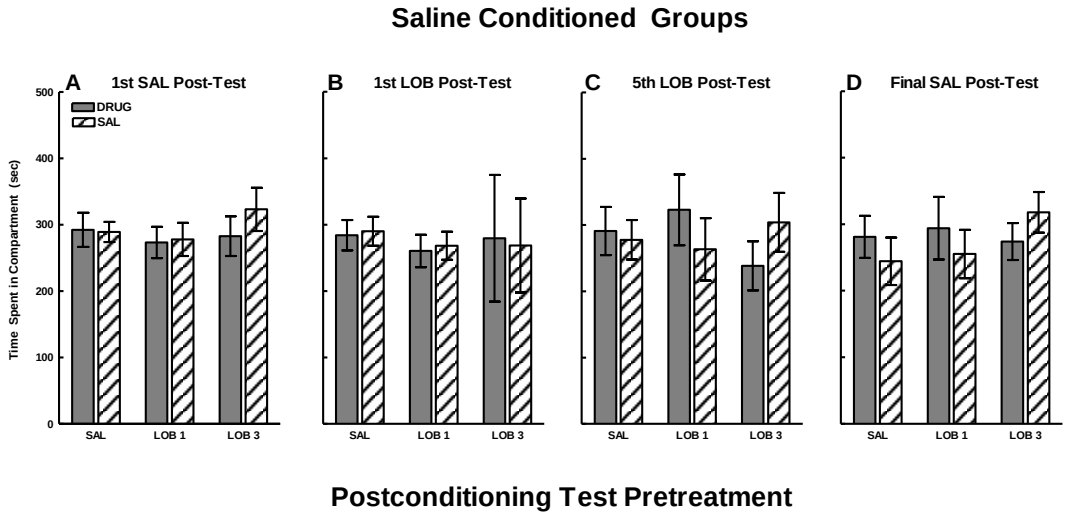


Figure 3.4 Expression of CPP in Rats that Received Saline During the Conditioning Phase. Mean (± SEM) amount of time spent in the saline- and drug-paired compartments in the saline conditioned control group pretreated with lobeline (0, 1 or 3 mg/kg) 15 min prior to 15-min postconditioning test sessions. **Panel A-D**: No group exhibited CPP during any of the postconditioning test sessions.

However, in the methamphetamine (0.5 mg/kg) conditioned groups (Figure 3.5) the analysis revealed a main effect of compartment ($F(1,33)=21.33$; $p<0.01$). Planned comparisons that revealed all groups conditioned with methamphetamine (0.5 mg/kg) spent significantly more time in the drug paired compartment on the 1st saline and final saline postconditioning tests, indicating a persistent methamphetamine-induced CPP across the repeated test days. The lower dose of lobeline (1 mg/kg) did not attenuate this response on the 1st lobeline postconditioning test. Interestingly, repeated administration of this dose did

attenuate the expression of CPP on the 5th lobeline postconditioning test ($p<0.05$). The higher dose of lobeline (3 mg/kg) attenuated the expression of CPP on both the 1st and 5th lobeline postconditioning tests ($p<0.05$).

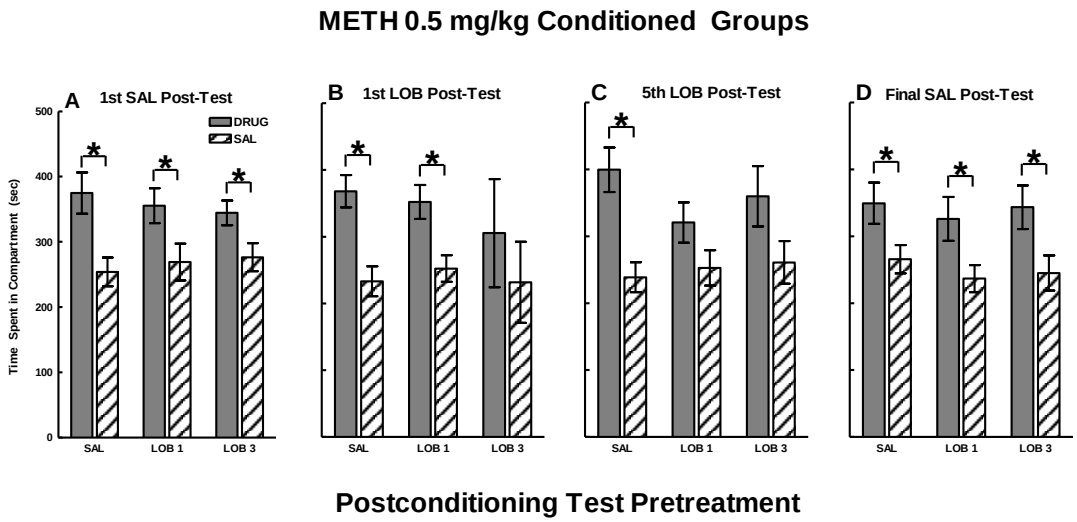


Figure 3.5 Expression of CPP in Rats that Received Methamphetamine (0.5 mg/kg) During the Conditioning Phase. Mean (± SEM) amount of time spent in the saline- and drug-paired compartments in treatment groups conditioned with methamphetamine (0.5 mg/kg) and pretreated with lobeline (0, 1 or 3 mg/kg) 15 min prior to 15-min postconditioning test sessions. **Panel A**: All treatment groups showed significant acquisition of methamphetamine CPP. **Panel B:** Acute lobeline (3 mg/kg) pretreatment blocked the expression of methamphetamine (0.5 mg/kg)-induced CPP. **Panel C:** Repeated lobeline (1 or 3 mg/kg) pretreatment blocked the expression of methamphetamine (0.5 mg/kg)-induced CPP. **Panel D**: All treatment groups showed persistent expression of methamphetamine CPP on the final saline challenge day. Asterisk (*) indicates a significant difference in time spent in the saline versus drug paired compartment; $p<0.05$.

In the methamphetamine (1.5 mg/kg) conditioned groups (Figure 3.6), the analysis revealed a main effect of compartment ($F(1,32)=18.20$; $p<0.01$), as well as an interaction of compartment x test day ($F(3,96)=2.80$; $p<0.05$). Planned comparisons revealed that rats conditioned with methamphetamine (1.5 mg/kg) and given a saline injection on the 1st and final saline postconditioning tests showed

significant CPP, indicating a persistent methamphetamine-induced CPP across test days. The lower dose of lobeline (1 mg/kg) did not attenuate this response on the 1st lobeline postconditioning test, but repeated administration of this dose did attenuate the expression of CPP on the 5th postconditioning test ($p<0.05$). However, the higher dose of lobeline (3 mg/kg) attenuated the expression of CPP on both the 1st and 5th lobeline postconditioning tests ($p<0.05$). In addition, the lobeline (1 and 3 mg/kg) pretreatment groups did not show significant conditioned place preference on the final saline postconditioning test.

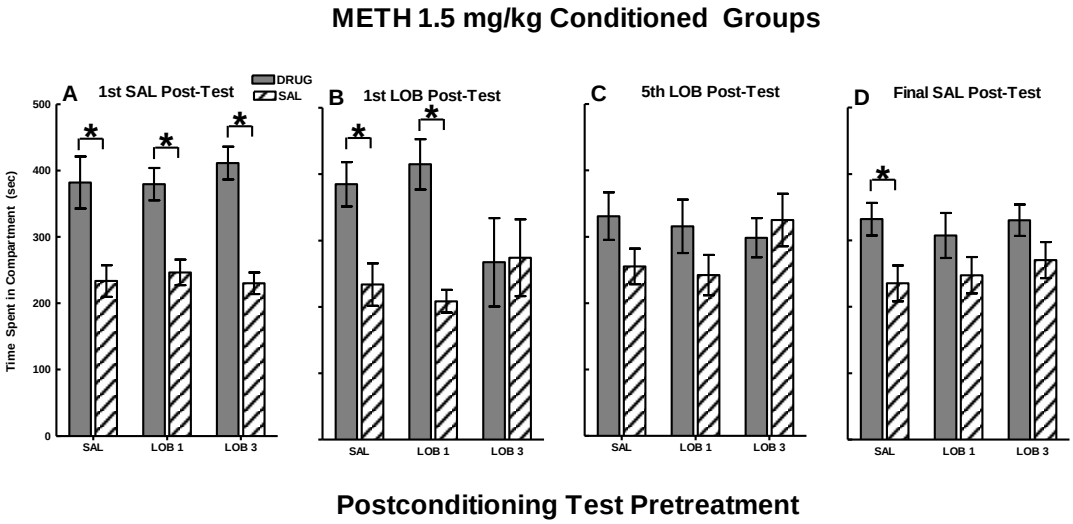


Figure 3.6 Expression of CPP in Rats that Received Methamphetamine (1.5 mg/kg) During the Conditioning Phase. Mean (± SEM) amount of time spent in the saline- and drug-paired compartments in treatment groups conditioned with methamphetamine (1.5 mg/kg) and pretreated with lobeline (0, 1 or 3 mg/kg) 15 min prior to 15-min postconditioning test sessions. **Panel A**: All treatment groups showed significant acquisition of methamphetamine CPP. **Panel B:** Acute lobeline (3 mg/kg) pretreatment blocked the expression of methamphetamine (0.5 mg/kg)-induced CPP. **Panel C:** Repeated lobeline (1 or 3 mg/kg) pretreatment blocked the expression of methamphetamine (0.5 mg/kg)-induced CPP. **Panel D**: Repeated lobeline (1 or 3 mg/kg) pretreatment resulted in extinction of CPP on the final saline postconditioning test. Asterisk (*) indicates a significant difference in time spent in the saline versus drug paired compartment; $p<0.05$.

No significant correlation was found between locomotor activity and expression of CPP when all conditioning groups were included on the 1st SAL (r =

.15, n=101, n.s.), 1st LOB (*r* = .09, n=101, n.s.), 5th LOB (*r* = .19, n=101, n.s.) or Final SAL (*r* = .04, n=101, n.s.) postconditioning tests. Correlations conducted on each of the experimental groups at each postconditioning test day indicated no correlation existed between locomotor activity and expression of CPP.

Summary of Experiment 2

The aim of the current experiment was extend the findings presented in Experiment 1 by assessing the effects of lobeline on expression of methamphetamine-induced CPP. During the repeated postconditioning sessions, rats were administered saline or lobeline (1 or 3 mg/kg) 15 min prior to being placed in the CPP apparatus where they had access to all 3 compartments. Horizontal beam breaks were recorded for the 15 min test session. No differences between conditioning groups were observed on the first saline postconditioning test, indicating no conditioned hyperactivity in the methamphetamine conditioned groups. Regardless of conditioning group, significant decreases in locomotor activity were observed across the postconditioning sessions in rats that received saline or lobeline (1 mg/kg). However, these differences were small and likely due to habituation to the testing apparatus. Interestingly, lobeline (3 mg/kg) induced hypoactivity across all conditioning groups. While tolerance to this hypoactivity was evident following 5 repeated administrations, significant hypoactivity compared to saline was still observed. The expression of CPP was not correlated with locomotor activity, indicating that the expression of CPP is not likely masked by locomotor suppression.

In rats conditioned with the lower dose of methamphetamine (0.5 mg/kg), all groups spent significantly more time in the previously drug versus saline-paired compartment on the first saline postconditioning test, indicating significant acquisition of CPP. Moreover, the methamphetamine (0.5 mg/kg) conditioned groups, regardless of postconditioning test pretreatment, maintained significant CPP across postconditioning tests, demonstrating the persistence of CPP across the experimental regimen. Acute administration of the lower dose of lobeline (1 mg/kg) did not attenuate the expression of CPP (1st LOB Post-Test). However, by the 5th lobeline administration, a significant attenuation was observed, indicating differences in acute and repeated effects of this lower dose of lobeline. In addition,

acute and repeated administration of the higher dose of lobeline (3 mg/kg) resulted in a persistent blockade in expression of CPP.

Following conditioning with the higher dose of methamphetamine (1.5 mg/kg), all groups showed significant acquisition of CPP. In contrast to the methamphetamine (0.5 mg/kg) conditioned groups, the methamphetamine (1.5 mg/kg) conditioned groups showed less persistent CPP. In rats that received saline across all postconditioning tests, significant expression of CPP on the 5th lobeline postconditioning test was not observed. It is not clear why significant CPP was not observed on this day, but was again evident on the final saline postconditioning test. In addition, neither lobeline (1 or 3 mg/kg) pretreatment group demonstrated significant CPP on the final saline postconditioning test. Similar to the effects observed in the methamphetamine (0.5 mg/kg)-conditioned group, acute administration of the lower dose of lobeline (1 mg/kg) had effect no on the expression of CPP, but attenuation was evident following repeated administration. In addition, this group did not show persistent CPP on the final saline postconditioning test. Acute and repeated administration of the higher dose of lobeline (3 mg/kg) blocked expression of CPP and suppressed locomotor activity on the 1st and 5th lobeline administration. However, this group did not show significant CPP on the final saline postconditioning test. Contrary to the hypothesis, these results demonstrate that repeated administration of lobeline decreases expression of methamphetamine-induced CPP. It is not clear why the expression of CPP was persistent across repeated postconditioning tests when a low dose of methamphetamine (0.5 mg/kg) was, but not when a high dose (1.5 mg/kg) was used. It is unlikely that the absence of CPP in the methamphetamine (1.5 mg/kg) conditioned groups that received repeated lobeline is due to non-specific extinction of CPP as groups pretreated with saline during the repeated postconditioning-tests continued to display significant CPP on the final test day. In addition, previous work has shown that methamphetamine CPP is resistant to extinction when rats are given repeated postconditioning tests for 10 consecutive days (Bahi, Kusnecov, & Dreyer, 2008).

CHAPTER FOUR

Experiment 3

Lobeline Alters Methamphetamine-Induced Changes in the Nucleus Accumbens Shell

The nucleus accumbens shell is a neuroanatomical area that has been implicated in playing a vital role in mediating the reinforcing effects of psychostimulants. Dopaminergic mechanisms in this brain region have been shown to be particularly important in the circuitry underlying drug-taking behaviors. Since lobeline has been shown to attenuate behaviors associated with the reinforcing and rewarding aspects of methamphetamine, it is of interest to assess the effects of lobeline on extracellular dopamine in this brain region. In addition, the mechanism by which lobeline disrupts methamphetamine-induced alterations in dopamine release has been assessed solely at the *in vitro* level, so determining if this neurochemical effect is also observed *in vivo* using microdialysis in an awake and behaving animal is warranted. Additionally, extracellular levels of DOPAC were assessed to determine if systemic lobeline administration results in an increased level of extracellular DOPAC, as lobeline is thought to increase the amount of cytosolic dopamine available for metabolism (Dwoskin & Crooks, 2002). It was hypothesized that lobeline would attenuate methamphetamine-induced alterations in extracellular dopamine and DOPAC in the nucleus accumbens shell.

Methods

Subjects

Male Sprague-Dawley rats (N=32; 225-250 g) were obtained from Harlan Industries (Indianapolis, IN) and housed one per cage. In all other respects, rats were cared for as described in Experiment 1.

Drugs and Chemicals

Ketamine (80 mg/kg, IP) and diazepam (5 mg/kg, IP) were be used as anesthetics during surgical procedures. All other drugs were the same as described

in Experiment 1. All reagents for the aCSF, HPLC mobile phase were obtained from Sigma (St. Louis, MO).

Surgery

Animals were anesthetized and implanted with guide cannula (secured with dental acrylic) aimed at the nucleus accumbens using the following coordinates relative to bregma: AP +1.6 mm, L +0.8 mm, and D/V -5.8 mm (Paxinos & Watson, 1986). Guide cannula (20 gauge; MD-2251) and probes (2mm; MD-2200) were obtained from BAS (Indianapolis, Indiana).

In Vivo Microdialysis

Microdialysis experiments were conducted using a swivel system (BAS) attached to the side of a Plexiglass chamber (25 x 44 x 38 cm), which contained pine chip bedding. Rats were assigned randomly to one of 6 different treatment groups making up a 3 x 2 factorial design (Table 2). The day before the microdialysis session, each animal was fitted with a plastic collar. The next day, rats were weighed and habituated to the plexiglass chamber for at least 30 min. The microdialysis probe, which was connected to a microsyringe pump (KD Scientific, Model KDS250) via PE10 tubing that was slowing perfusing artificial cerebral spinal fluid (aCSF; consisting of: 145 mM sodium chloride, 2.7mM potassium chloride, 1 mM magnesium chloride, 1.2 calcium chloride, and 2.0 mM sodium phosphate) through the probe at a flow rate of 1.2 µl/min, was inserted into the guide cannula and the animal was connected to the swivel system by attaching a leash to the collar. The rats were then habituated to the Plexiglas chamber and probe insertion for at least 3.5 hr prior to collection of the baseline samples. Baseline samples were collected into polyethylene microfuge tubes containing 5 µl of 0.1 N perchloric acid every 20 min for 60 min. After collection of 3 baseline samples, each rat was administered either saline or lobeline (1 or 3 mg/kg, SC) and 5 min later injected with saline or methamphetamine (0.5 mg/kg, IP).

Table 4.1 Experimental groups in Experiment 2.

Pretreatment (Inj 1)	Treatment (Inj 2)	n
SAL	SAL	4
SAL	METH 0.5 mg/kg	6
LOB 1 mg/kg	SAL	6
LOB 1 mg/kg	METH 0.5 mg/kg	7
LOB 3 mg/kg	SAL	6
LOB 3 mg/kg	METH 0.5 mg/kg	7

Dialysis samples were collected every 20 min for an additional 3 hr after the second injection. Samples were frozen immediately on dry ice and stored at -70˚ C for later analysis. Following the microdialysis experiment, the brains were removed and flash frozen in Chromasolv® (Sigma). Brains were sectioned into 40 µm coronal slices, mounted onto slides and stained with cresyl violet. Microdialysis probe placement in the nucleus accumbens was confirmed as indicated by Paxinos and Watson (1986) and only data from rats with confirmed probe placement were included.

Analysis of Extracellular Dopamine and DOPAC using HPLC-EC

Samples were thawed and analyzed immediately for dopamine and DOPAC (3,4-dihydroxy-phenylacetic acid) using HPLC-EC (ESA Chelmsford, MA, USA) as previously described (Rahman et al., 2003). The system consisted of a computer running EZ-Chrome Elite software, a solvent delivery system (ESA pump 582), a 3 µm, C18 column with guard column, a Coulochem III 5200A electrochemical detector and manual injector equipped with an ESA 5011 analytical cell and 5020 guard cell. The guard cell was set at 225 mV, the reference electrode on at –150mV and the working electrode was 225mV. The gain was set to 1µ A and changed to 10 nA at 4.5 min in order to assess both DOPAC and DA in the same sample. The mobile phase consisted of: 75 mM NaH_2PO_4, 1.7 nM 1-octanesulfonic acid, 25 µM EDTA, 100 µl/l triethylamine and 10 % acetonitrile; pH 3.0 adjusted with phosphoric acid, and pumped through the system at a rate of 0.65 ml/min. Samples were loaded into a 20 µl sample loop and manually injected onto an analytical column (BetaBasic-18 column, 150 mm x 3mm; Keystone Scientific, PA, USA).

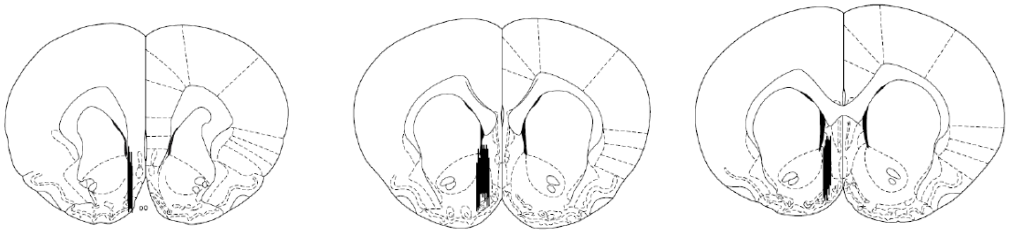
External standards were used to determine the actual concentrations of dopamine and DOPAC in each sample.

Data Analysis

Data were recorded as peak height for DOPAC and dopamine for each sample collected. These data were then expressed as a percent of baseline (average of the 1st three samples) and analyzed with SPSS (Chicago, IL) software using 2-way repeated measures ANOVA (Treatment Group x Time). In addition, area under the curve was calculated for each experimental group and analyzed with a one way ANOVA across treatment group. Post-hoc analyses for between-subject effects were conducted using unpaired t- tests and paired t-tests with correction for family wise error were used to compare within-subject data points.

Results

Histology



A/P: 1.6 A/P: 1.7 A/P: 2.2

Figure 4.1 Placement of Microdialysis Probes. Each vertical bar represents an animal that was included in the analysis of the microdialysis experiment.

Analysis of Extracellular Dopamine in the Nucleus Accumbens Shell

The mean (± SEM) basal dopamine concentration was 0.60 ± 0.05 nM and the basal DOPAC concentration was 471 ± 24 nM. An overall analysis of dopamine levels using a 5 (treatment group) x 12 (time) mixed factor ANOVA with treatment group as a between subject variable and time as a within subject variable indicated significant main effects of group ($F(4,27)=4.54$; $p<0.01$) and time ($F(11,297)=24.74$; $p<0.01$). This analysis also indicated a significant group x time interaction ($F(44,297)=4.27$; $p<0.01$). Posthoc analysis indicated that the groups receiving methamphetamine (0.5 mg/kg) showed a significant increase in dopamine levels

compared to baseline (Figure 4.2; p<0.05). Analysis of the area under the curve for each group indicated a significant groups effect (F(4,27)=5.25;p<0.01). Posthoc analysis indicated none of the methamphetamine treatment groups were significantly different from each other. Interestingly, lobeline had no effect on the methamphetamine-induced increase in dopamine, nor did it have any effect when administered prior to saline.

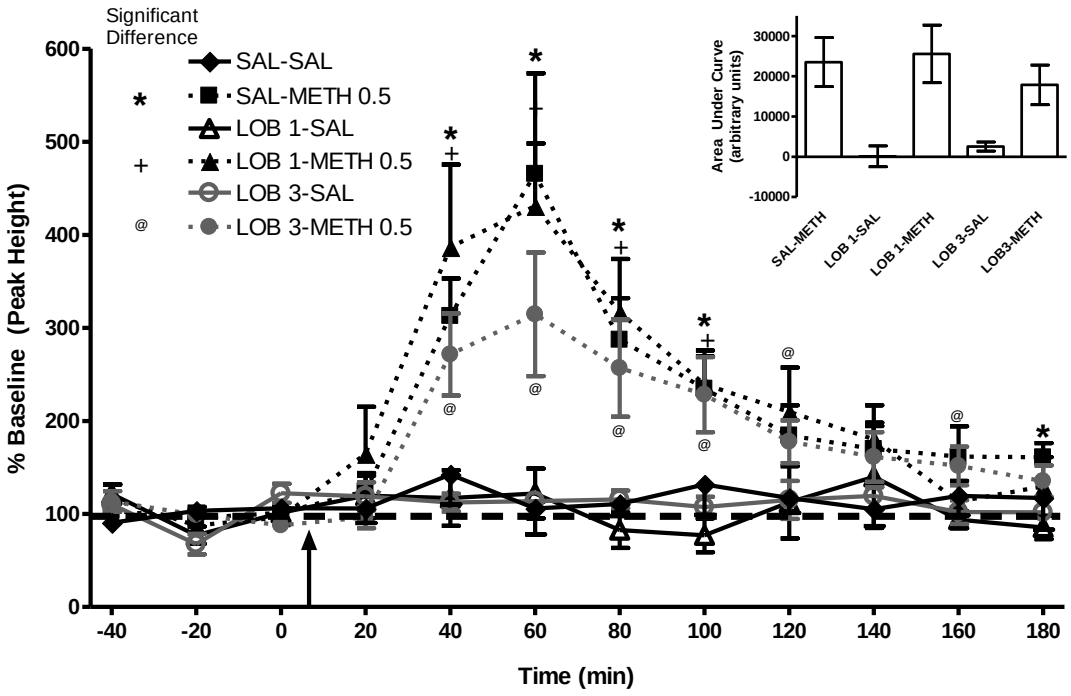


Figure 4.2 Extracellular Dopamine Levels in the Nucleus Accumbens Shell. Mean (± SEM) percent baseline of the dopamine peak height for each time point following administration of lobeline (LOB) and/or methamphetamine (METH). The arrow indicates time of treatment. Symbols indicate differences from the respective group's baseline. The thick dashed line represents the baseline. The insert is the calculated area under the curve for each group.

Analysis of Extracellular DOPAC in the Nucleus Accumbens Shell

An overall analysis of DOPAC levels using a 5 (treatment group) x 12 (time) mixed factor ANOVA, with treatment group as a between subject variable and time as a within subject variable, indicated significant main effects of group (F(4,27)=11.21; p<0.01) and time (F(11,297)=8.91; p<0.01). This analysis also indicated a significant group x time interaction (F(44,297)=7.96; p<0.01). Posthoc analysis

indicated that the groups receiving methamphetamine (0.5 mg/kg) showed a significant decrease in DOPAC levels compared to baseline (Figure 4.3; $p<0.05$). Furthermore, lobeline administration significantly enhanced this decrease in a dose-dependent manner ($p<0.05$). Analysis of the area under the curve for each group indicated a significant groups effect ($F(4,27)=9.14$; $p<0.01$). Posthoc analysis indicated none of the methamphetamine treatment groups were significantly different from each other. Interestingly, while lobeline enhanced the methamphetamine-induced decrease in DOPAC, it increased DOPAC levels dose-dependently when administered alone ($p<0.05$).

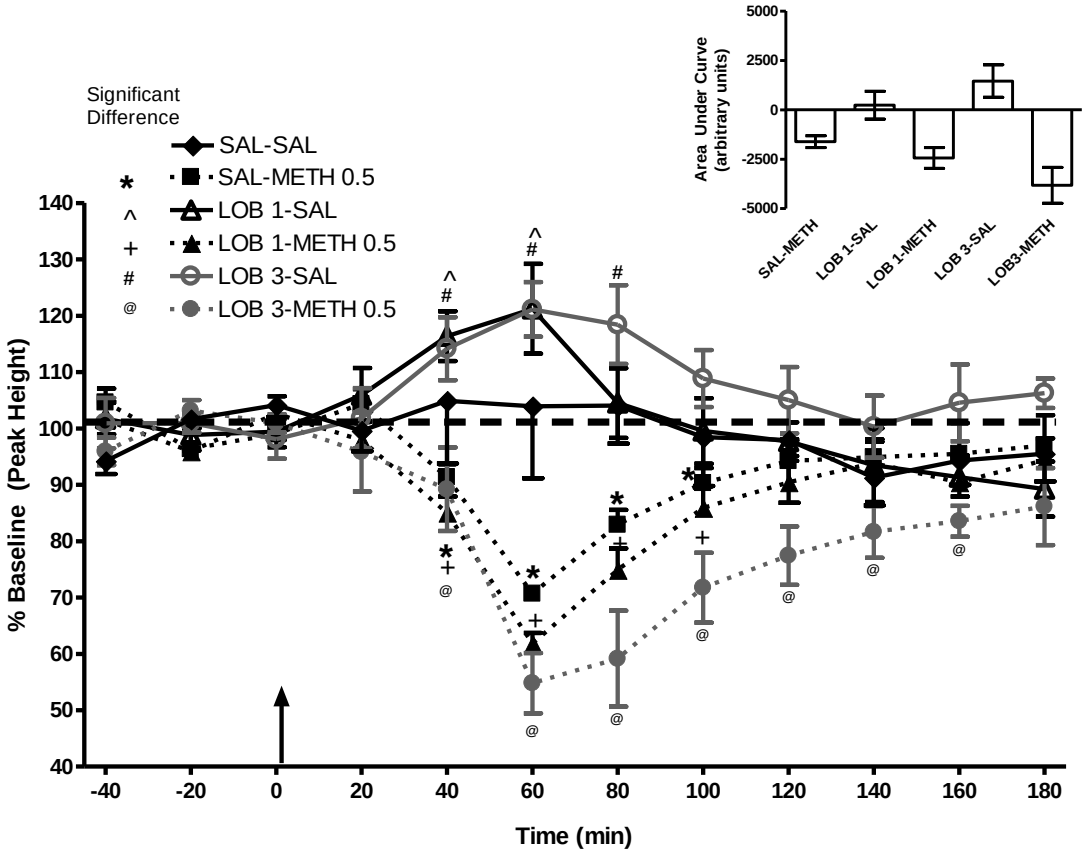


Figure 4.3 Extracellular DOPAC Levels in the Nucleus Accumbens Shell. Mean (± SEM) percent baseline of the DOPAC peak height for each time point following administration of lobeline (LOB) and/or methamphetamine (METH). The arrow indicates time of treatment. Symbols indicate differences from the respective group's baseline. The thick dashed line represents the baseline. The insert is the calculated area under the curve for each group.

Summary of Experiment 3

Microdialysis was conducted in awake, freely moving rats with probe placements in the nucleus accumbens shell. Methamphetamine administration increased extracellular levels of dopamine in this brain region to ~ 450% of baseline, while lobeline alone had no effect. Contrary to our hypothesis, pretreatment with either dose of lobeline (1 or 3 mg/kg) did not significantly alter the methamphetamine-induced increase in dopamine. However, a slight attenuation following the higher dose of lobeline (3 mg/kg) at 60 min was noted. Interestingly, lobeline alone increased DOPAC (~20%) at both doses tested, indicating an increase in dopamine metabolism. As expected, methamphetamine alone decreased DOPAC by ~30%, indicating a decrease in dopamine metabolism. Lobeline pretreatment dose dependently enhanced the methamphetamine-induced decrease, indicating synergistic inhibition of dopamine metabolism when lobeline was administered prior to methamphetamine. Specifically, lobeline (1 and 3 mg/kg) pretreatment prior to methamphetamine induced a more pronounced decrease in DOPAC at 60 min than methamphetamine alone. Furthermore, the higher dose of lobeline (3 mg/kg) combined with methamphetamine decreased extracellular DOPAC for a longer period of time than methamphetamine alone.

CHAPTER FIVE
General Discussion

The overall hypothesis of the current experiments was that lobeline would block the acquisition and expression of methamphetamine-induced CPP and that this behavioral effect would be associated with an inhibition of methamphetamine-induced dopamine release in the reward-relevant nucleus accumbens shell. Previous behavioral research has indicated that lobeline is effective at attenuating self-administration in rats, a behavior that is acquired and maintained through operant conditioning. In the current studies, the effects of lobeline on methamphetamine-induced behaviors that are learned through classical conditioning were assessed. Through this type of learning, environmental cues that were previously predictive of drug reward come to elicit a conditioned response, which is thought to contribute significantly to context-dependent relapse in humans.

Few studies have examined the effects of low doses of lobeline (≤ 1 mg/kg) following repeated administration. This dose of lobeline does not result in the non-specific suppression of activity as is observed following the higher doses. For example, lobeline (1 mg/kg) does not acutely decrease response rates in the drug discrimination paradigm in animals trained to discriminate nicotine or methamphetamine nor does this dose decrease operant responding for methamphetamine or food reinforcement (Damaj, Patrick, Creasy, & Martin, 1997; Harrod et al., 2001; Miller et al., 2003). However, this dose of lobeline is behaviorally activity as it produces a conditioned taste aversion to a salt solution and has been shown to decrease the progressive ratio breakpoint for intracranial self-stimulation in rats (Harrod, Dwoskin, & Bardo, 2004; Wellman et al., 2008). This latter study also suggests that lobeline is not reinforcing as drugs of abuse increase the progressive ratio breakpoint for intracranial self-stimulation in rats. The current experiments are the first to demonstrate that lobeline (1.0 mg/kg) also attenuates methamphetamine-induced reward and future studies assessing the repeated effects of lower doses of lobeline are warranted.

Following acute administration of lobeline (≥ 3 mg/kg), a non-specific decrease in activity is often observed. Tolerance to these non-specific effects is generally evident within ~5-7 repeated administrations. In addition to locomotor

hypoactivity, as was observed in the current studies, higher doses of lobeline acutely decrease response rates in drug discrimination and operant responding for food (Harrod et al., 2001; Miller et al., 2001). Little is known about the mechanism(s) underlying the lobeline-induced decrease in activity. Currently, there are no known pharmacological manipulations that will block this hypoactivity following lobeline (≥ 3 mg/kg), making it difficult to know with certainty that acute administration of these doses decreases methamphetamine reward specifically and is not simply disrupting ongoing behavior in a non-specific manner.

Lobeline, which is a nicotinic receptor ligand, is known to reliably produce emesis or nausea in humans and this illness-inducing effect may explain the decrease in behavior observed in rats (Dwoskin & Crooks, 2002). Interestingly, while nicotine also produces a non-specific decrease in behavior when administered acutely, the non-specific, non-selective, nicotinic receptor antagonist mecamylamine blocks nicotine-induced hypoactivity, while having no effect on lobeline-induced hypoactivity (Damaj et al., 1997). This suggests that while acute nicotine and lobeline administration produce a similar non-specific decrease in behavior, they do so via different pharmacological mechanisms.

Research has suggested that neuronal nicotinic receptors modulate amphetamine-induced behaviors. Antagonism of nicotinic receptors with mecamylamine prior to repeated amphetamine administration attenuates amphetamine-induced locomotor sensitization; interestingly, after amphetamine sensitization is established, mecamylamine has no effect on amphetamine-induced hyperactivity (Schoffelmeer, De Vries, Wardeh, Van De Ven, & Vanderschuren, 2002). This study suggests neuronal nicotinic receptors modulate amphetamine-induced neuronal alterations. Since lobeline is known to act as a nicotinic receptor antagonist, it is possible that this mechanism contributes to the lobeline-induced disruption of methamphetamine reward. It is unlikely that antagonism of nicotinic receptors alone is responsible for the disruption of methamphetamine-induced CPP in the current study, as mecamylamine administration does not affect acquisition of methamphetamine self-administration, nor does it disrupt fully acquired methamphetamine self-administration (unpublished results from our laboratory). Evidence suggests that nicotinic receptor activity may be an important mechanism for the persistence of the effect of lobeline on methamphetamine reward. This

evidence is based on work showing that lobelane, an analog of lobeline, selectively and potently inhibits the vesicular monoamine transporter and the dopamine transporter more potently than lobeline (Miller et al., 2004). However, lobelane interacts with the nicotinic receptors less potently than lobeline (Miller et al., 2004). Similar to lobeline, lobelane was shown to acutely decrease methamphetamine self-administration (Neugebauer et al., 2007). However, rapid tolerance developed to the lobelane-induced decrease in methamphetamine self-administration upon repeated administration, suggesting that nicotinic receptor binding may be necessary for the persistent decrease in methamphetamine self-administration observed following repeated lobeline. Further understanding of the role that nicotinic receptors play in psychostimulant-induced behaviors is necessary to fully characterize the contribution this mechanism may have on the persistent nature of lobeline-induced decreases of methamphetamine-induced behaviors.

Lobeline may be acting to decrease acquisition of methamphetamine-induced CPP by inducing disruptions in the mechanisms involved in Pavlovian learning. It is currently unknown if lobeline affects these mechanisms. One would expect that if lobeline (3 mg/kg) were impairing the formation of environmental and drug reward associations then this dose of lobeline would block the acquisition at all methamphetamine conditioning doses, not just the 0.5 mg/kg dose as demonstrated in experiment 1. Since this was not the case, it is unlikely that the results obtained here reflect merely a disruption in Pavlovian learning.

Taken together, the results from the present behavioral experiments demonstrate that lobeline has differential effects on the acquisition and expression of methamphetamine-induced CPP, as well as methamphetamine-induced locomotor hyperactivity. Lobeline (1 and 3 mg/kg) blocked the acquisition of CPP when given in combination with a low dose of methamphetamine (0.5 mg/kg) during the conditioning phase. While only the higher dose of lobeline (3 mg/kg) acutely blocked the expression of CPP, a blockade was observed following repeated administration of both doses (1 and 3 mg/kg). As discussed in the introduction, amphetamine CPP is not dependent on locomotor activity (i.e. restrained rats will acquire CPP). However, the effect of locomotor hypoactivity during postconditioning tests is not entirely clear. It is unlikely that the hypoactivity contributes significantly to the disruption of CPP as a correlational analysis

indicated that no relationship exists between locomotor activity and conditioned place preference during the postconditioning tests

While both psychostimulant-induced reward and locomotor hyperactivity are dependent on mesolimbic dopamine pathways, they appear to be due to independent processes (Carr et al., 1988). Dissociation between conditioned reward and locomotor hyperactivity has been observed previously with amphetamine and these behaviors may be regulated by anatomically distinct regions of the nucleus accumbens (Sellings & Clarke, 2003). Sellings and Clarke (2003) assessed rats with bilateral 6-hydroxydopamine lesions of the nucleus accumbens core or shell on CPP acquisition, CPP expression and locomotor activity induced with amphetamine (0.75 mg/kg). Their results indicate that the nucleus accumbens shell meditates amphetamine-induced reward, as lesions in this area inhibited acquisition and expression of amphetamine-induced CPP, while having no effect on amphetamine-induced locomotor hyperactivity. In addition, lesions of the nucleus accumbens core did not disrupt acquisition or expression of amphetamine-induced CPP, but did attenuate amphetamine-induced locomotor hyperactivity.

The nucleus accumbens shell has been implicated in reward-related behaviors. Previous research has shown that rats will self-administer amphetamine directly into this brain region (Hoebel et al., 1983). In addition, intracranial administration of amphetamine into this brain region results in CPP (McBride et al., 1999; Schildein, Agmo, Huston, & Schwarting, 1998). Furthermore, the rewarding effects of amphetamine can be blocked by 6-hydroxydopamine lesions of the nucleus accumbens shell, as well as local administration of D1 or D2 antagonists (Hoffman & Beninger, 1989; Spyraki et al., 1982). These studies indicate that intact dopaminergic mechanisms in the nucleus accumbens shell are critically important for amphetamine-induced reward. Based on these results, we hypothesized that the effects observed in the current study are a result of lobeline blocking the amphetamine-induced increase of extracellular dopamine levels in the nucleus accumbens shell.

To test this hypothesis, we examined the effect of lobeline on methamphetamine-evoked dopamine release in the nucleus accumbens shell using *in vivo* microdialysis. Dopamine levels in the nucleus accumbens shell were

increased (~450%) following administration of a relatively low dose of methamphetamine (0.5 mg/kg, SC). In addition, a concomitant decrease in extracellular DOPAC (~30%) was evident. This dose of methamphetamine (0.5 mg/kg) does not cause neurotoxicity and is a weak inhibiter of monoamine oxidase (Miller, Shore, & Clarke, 1980). It has been suggested previously that the methamphetamine-induced decrease in DOPAC may be due to the redistribution of newly synthesized dopamine such that it is not available for monoamine oxidase degradation (Shimosato, Nagao, Watanabe, & Kitayama, 2003). In addition, methamphetamine-induced reversal of the dopamine transporter presumably results in less dopamine available for metabolism to DOPAC in the cytosol.

The current results demonstrate that lobeline (1 or 3 mg/kg) alone has no effect on extracellular dopamine levels in the nucleus accumbens shell, but does increase the extracellular concentration of DOPAC (~20%). These results coincide with previous findings indicating that administration of a high dose of lobeline (10 mg/kg) does not alter extracellular dopamine levels in striatum as assessed with *in vivo* microdialysis in awake rats (Eyerman & Yamamoto, 2005). Unfortunately, dose comparisons cannot be made with regard to DOPAC, as this was not reported in the study by Eyerman & Yamamoto (2005). However, another report using *in vivo* microdialysis to assess the effects of lobeline in nicotine pretreated rats in the nucleus accumbens core indicated lobeline alone (10 mg/kg, IP) did not affect extracellular dopamine or DOPAC (Benwell & Balfour, 1998). The reason for the discrepancy in results between the study by Benwell and Balfour (1998) and the current study may be due to neuroanatomical differences between the shell and core subregions on the nucleus accumbens (see above). Finally, when lobeline (2, 4, 6 nmol at a rate of 2 µl/min for 1 min) is perfused directly into the rat striatum via a microdialysis probe, a dose-dependent increase in extracellular dopamine is observed; however, DOPAC levels were not reported in this study (Lecca, Shim, Costa, & Javaid, 2000). Since little is known about the diffusion properties of lobeline, it is unclear whether the tissue concentrations achieved around the microdialysis probe in this latter study are comparable to those achieved via systemic administration. Differences in tissue concentrations of lobeline in the target region could account for the differences observed.

Previous research using an *in vitro* superfused striatal slice preparation has demonstrated that a low concentration of lobeline (1 µM) has little effect on dopamine release, while increasing DOPAC (Teng et al., 1997). However, higher concentrations (100 µM) of lobeline result in dopamine release (Teng et al., 1997). The dopamine release observed at the higher concentration is thought to occur when dopamine levels in the cytosol exceed the enzymatic capabilities of monoamine oxidase (Teng et al., 1997). Interestingly, pharmacokinetic data indicates that when a rat is administered lobeline (4.0 mg/kg; SC) the resulting brain concentration is 237 ng/ml (or ~0. 7 µM), indicating that the findings with the lower concentrations are more likely relevant for comparison to *in vivo* experiments which typically use lobeline doses between 0.3 – 10 mg/kg (Reavill, Walther, Stolerman, & Testa, 1990). Importantly, the brain concentration of lobeline achieved following systemic administration of 4.0 mg/kg is similar to the IC_{50} for lobeline inhibition of [^{3}H]DA uptake into vesicles (0.88 µM) *in vitro*, which is thought to be the primary mechanism responsible for the lobeline-induced decrease of methamphetamine-induced behaviors (Dwoskin & Crooks, 2002; Teng et al., 1997). The differences between *in vivo* and *in vitro* findings are probably due to differences in lobeline concentration, as the systemic lobeline doses administered in the current study do not result in a brain concentration high enough to increase extracellular dopamine levels.

It is well accepted that increases or decreases in extracellular DOPAC are, at least in part, the result of altered vesicular storage of dopamine (Eisenhofer, Kopin, & Goldstein, 2004). As described in the introduction, amphetamine and lobeline have been shown to interact with the vesicular monoamine transporter. It is likely that the lobeline induced increase in DOPAC observed in the current study is due to inhibition of dopamine uptake into vesicles, allowing for more cytosolic dopamine to be available for monoamine oxidase metabolism to DOPAC (Teng et al., 1998). In contrast, the decreases in DOPAC following methamphetamine in the current study are likely due to the redistribution of newly synthesized dopamine in such a manner that it is not physically available to monoamine oxide for metabolism (Zetterstrom, Sharp, Collin, & Ungerstedt, 1988). It is possible, albeit less likely, that the dose of methamphetamine used in the current study inhibits the activity monoamine oxidase. The ability of amphetamine to decrease monoamine oxidase activity is

observed following higher systemic doses than those administered in the current study (Miller et al., 1980).

No previous *in vivo* studies have reported that lobeline administration alone results in increased extracellular DOPAC, while simultaneously enhancing the methamphetamine-induced decrease. One possibility for this outcome is that lobeline may alter the methamphetamine-induced metabolism of DA to DOPAC in the cytoplasm; however, previous *in vitro* research indicates that lobeline does not inhibit monoamine oxidase (Miller et al., 2001). While the current studies do not address this question directly, it is unlikely that lobeline alters the enzymatic activity involved in metabolizing dopamine to DOPAC. During this process, monoamine oxidase deaminates dopamine to 3,4-dihydroxyphenylacetaldehype, which is then oxidized by aldehyde dehydrogenase to DOPAC (Eisenhofer et al., 2004). If lobeline were decreasing the enzymatic activity of either of these enzymes, a decrease in DOPAC would be expected. Likewise, an increase in the activity of these enzymes would be expected to result in an increase in DOPAC. It seems unlikely that lobeline would increase the activity of these enzymes when administered alone, but conversely decrease the activity of these enzymes in combination with methamphetamine. Thus, the current results suggest the effect of lobeline, as well as the effect of lobeline pretreatment prior to methamphetamine administration, is not due to interactions with enzymatic pathway responsible for converting dopamine to DOPAC.

As an alternative explanation, recent studies have suggested that psychostimulants can interact with proteins that help regulate and maintain the reserve and readily releasable dopamine stores (Venton et al., 2006). One possibility for the current microdialysis study results is that methamphetamine induces a redistribution of a portion of the readily-releasable vesicular stores of dopamine to a site within the cytosol that renders dopamine not sequestered in vesicles to be less likely to interact with monoamine oxidase. This would result in increased dopamine available for reverse transport. While there is currently no information available about interactions of lobeline with other vesicular proteins, perhaps methamphetamine changes the dynamics of lobeline's interactions with the vesicular membrane such that lobeline and amphetamine act synergistically to redistribute cytosolic dopamine stores. This could result in further decreases in

DOPAC due to a decrease of cytosolic dopamine in a region where monoamine oxidase has access to it. Thus, lobeline may act to redistribute cytosolic dopamine pools such that when methamphetamine is available, dopamine is unavailable for metabolism. The mechanisms responsible for these putative effects remain to be elucidated.

No significant effect of lobeline on the methamphetamine-evoked increase in extracellular dopamine was observed in the current experiment, although a slight decrease was noted at the 60-min time point. These results are consistent with findings reported by Eyerman and Yamamoto (2005) indicating that lobeline (10 mg/kg) does not acutely attenuate extracellular dopamine levels in the striatum following methamphetamine (10 mg/kg). In contrast, *in vitro* studies indicate that lobeline (0.3 and 1.0 μM) decreases amphetamine (1.0 μM) -evoked endogenous dopamine release in rat striatal tissue, indicating a discrepancy between *in vivo* and *in vitro* effects of lobeline (Miller et al., 2001). Additionally, a recent study using a human embryonic kidney cell system expressing isoforms of both the dopamine transporter and vesicular monoamine transporter found that lobeline (100 μM) decreases methamphetamine-evoked [^{3}H]DA release (Wilhelm, Johnson, Eshleman, & Janowsky, 2008). Differences among these studies in the effect of lobeline on methamphetamine-induced increases in dopamine levels likely reflect inherent differences between *in vivo* preparations using intact animals and *in vitro* preparations examining only a part of the neurocircuitry involved in the drug effects.

Since lobeline administration decreases methamphetamine-induced behaviors, it is possible that lobeline may enhance the peripheral metabolism of methamphetamine. While it is currently unknown if lobeline alters methamphetamine brain concentrations, the current results showing that lobeline does not alter methamphetamine-induced dopamine release in the nucleus accumbens shell argues against a potential pharmacokinetic interpretation. Based on such a pharmacokinetic interpretation, lobeline should have attenuated the methamphetamine-induced increase in extracellular dopamine levels.

Lobeline (3 mg/kg) pretreatment has been shown to decrease methamphetamine self-administration and conditioned place preference, which suggests that lobeline may be attenuating the effects of methamphetamine reinforced behavior via mechanisms other than extracellular dopamine levels in the

nucleus accumbens shell. Another possibility is that lobeline may decrease methamphetamine-evoked dopamine release more completely following repeated administration. To date, it can not be concluded with certainty that acute lobeline (3 mg/kg) is specifically decreasing methamphetamine reward, rather than having non-specific effects. Following repeated administration, lobeline (3 mg/kg) more specifically decreases methamphetamine self-administration and the expression of CPP, while having a decreased effect on other behaviors (i.e. responding for sucrose reinforcement or locomotor activity) Harrod et al., 2001). Thus, methamphetamine-evoked increases in extracellular dopamine levels may be decreased following repeated lobeline administration. In any case, the current studies emphasis the importance of assessing potential pharmacotherapies following repeated administration and including doses that do not affect behavior acutely.

Integration with Previous Work

Lobeline has been purposed to be a potential pharmacotherapy for psychostimulant addiction, attributable in part to its unique pharmacological profile (Dwoskin & Crooks, 2002). Elucidating the mechanisms by which lobeline reduces the rewarding effects of psychostimulants would aid in the development of pharmacotherapies for the treatment of drug dependence. It is unlikely that all of lobeline's pharmacological mechanisms are currently known and future research will surely uncover additional molecular targets with which lobeline interacts. Lobeline has been shown to reduce methamphetamine self-administration in rats, indicating it decreases the primary reinforcing effects of methamphetamine (Harrod et al., 2001). The current studies indicate that lobeline is also effective at attenuating the conditioned environmental cues associated with methamphetamine administration. In addition, the effects of lobeline on cocaine-induced behaviors have also been examined. Interestingly, lobeline decreases cocaine self-administration and acquisition of conditioned place preference. One could speculate that nicotinic receptor antagonism, such as that provided by lobeline, may be a common pharmacological target for both methamphetamine and cocaine. However, additional studies have indicated that mecamylamine does not alter the acquisition of methamphetamine self-administration nor does alter stable

methamphetamine self-administration. Taken together, these experiments suggest that lobeline is likely decreasing psychostimulant-induced behaviors by altering the transport vesicular dopamine stores within the presynaptic terminal. Since both methamphetamine self-administration and conditioned place preference were attenuated by lobeline, these results suggest that both the primary rewarding and the secondary cue-elicited rewarding effects of methamphetamine involve VMAT2.

Limitations

Locomotor activity is a commonly reported behavioral measure of psychostimulant-induced behavior. While the automation of many various types of behavioral testing apparatuses has provided efficient data collection, it is unclear how sensitive each of these automated indices are to different drug treatments. For example, in Experiment 1, lobeline did not cause hypoactivity compared to controls following the 4th administration of lobeline when animals were confined to one compartment (last conditioning day). However, hypoactivity was still observed following the 5th administration of lobeline when animals were allowed access to the entire chamber (postconditioning tests). Since lobeline has been shown to be behaviorally active for ~30 min, these differences in observed locomotor activity may be due to the differences in test session length, as the conditioning sessions were 30 min and the postconditioning tests were 15 min (Harrod et al., 2001). While it is informative to have locomotor activity data during CPP experiments, close examination of the testing apparatus and time course effects are necessary for comparisons across studies.

The dose of methamphetamine used for the microdialysis study is on the low end of its dose effect curve for most behaviors and results from our laboratory suggest that 0.5 mg/kg of methamphetamine is a threshold dose for acquisition of CPP. While it appears that lobeline may have attenuated methamphetamine-evoked dopamine release, this effect failed to reach significance. Upon closer examination of these groups, it is evident that there was greater variability in the peak effects of the methamphetamine-induced increase in dopamine than in the lobeline alone groups. The effects of lobeline may have been masked due to the variability in response to methamphetamine at this dose. One likely contributing factor for the variability relates to the probe placement. Although all probes were in

the nucleus accumbens shell, portions of the probe extended beyond the shell. Smaller probes and stricter criteria for data inclusion based on probe placement may be useful for reducing this variability.

The current study did not address the possibility that acquisition of lobeline CPP may be state dependent. State dependent learning is the phenomenon in which expression of a learned behavior occurs only when an organism is in the same physiological or contextual state during recall as it was during learning (Overton, 1991). It cannot be ruled out that lobeline alone is not rewarding in this paradigm, as the learned association between reward and environmental cues may be state-dependent. In order to assess this, rats given lobeline during the conditioning phase would need to be given lobeline prior to the postconditioning test. It is possible that lobeline CPP would be evident only when rats are in the same drug state as they were during conditioning. Interestingly, like in the current study, previous assessment of lobeline-induced CPP did not address the issue, indicating that more work is needed (Fudala & Iwamoto, 1986).

Future Directions

Recent evidence suggests that, in addition to its effect on dopaminergic mechanisms, lobeline may function as a mu opioid receptor antagonist (Miller et al., 2007). The mu-opioid system is thought to play a role in cue-induced drug-seeking behavior, as assessed using cue and drug-primed reinstatement of methamphetamine seeking behavior in rats, as well as using sensitization to methamphetamine (Anggadiredja, Sakimura, Hiranita, & Yamamoto, 2004). In addition, mu-opioid receptor antagonists have been shown to decrease amphetamine-induced increases in dopamine levels in the nucleus accumbens (Chiu, Ma, & Ho, 2006; Schad, Justice, & Holtzman, 1996). It is currently unknown if mu opioid antagonists decrease rodent amphetamine self-administration when given repeatedly; however, there is some indication that the opiate antagonist naltrexone may be useful as an adjunct pharmacotherapy for amphetamine dependence in humans (Jayaram-Lindstrom, Wennberg, Beck, & Franck, 2005). Further characterization of lobeline's ability to attenuate the psychoactive effects of opiates and how this pharmacological action may interact with methamphetamine conditioned reward is warranted.

As suggested previously, the effects of repeated administration of lobeline on methamphetamine-induced behaviors warrants further investigation. While previous studies have shown that lobeline does not attenuate reinstatement of methamphetamine-seeking specifically, only acute effects of this dose were assessed (Harrod et al., 2003). Since repeated administration of lobeline blocked the expression of CPP, it is of interest to assess the effects of repeated lobeline on drug and cue-induced reinstatement in the operant paradigm. The current results suggest that repeated administration of lobeline may be more efficacious in attenuating reinstatement.

In summary, lobeline has many pharmacological actions that may contribute to its potential usefulness as a pharmacotherapy for psychostimulant dependence. It may be that a combination of pharmacological actions is necessary for the decrease observed in methamphetamine behaviors following lobeline administration. In addition, while self-administration is a standard procedure used to assess the direct reinforcing properties of drugs of abuse, differential results may be observed when assessing potential pharmacotherapies using the CPP paradigm. Perhaps pharmacotherapies that interact with a combination of molecular targets may be more effective than highly specific pharmacotherapies in attenuating the primary and secondary reinforcing properties of methamphetamine.

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VITA

Name: Nichole Marie Neugebauer

Date of birth: August 25, 1979

Place of birth: Parkston, South Dakota

Education:

8/02-present University of Kentucky at Lexington
Master of Science in Psychology, May 2005

8/98-5/01 University of Massachusetts at Boston
Bachelor of Science in Psychology
Minor in Biology
Magna Cum Laude, May 2001

1/97-5/98 University of South Dakota at Vermillion
Major: Psychology

Professional Positions:

8/02-8/08 University of Kentucky, Lexington, KY
Graduate Student/Research Assistant with Dr. M.T. Bardo in Behavioral Pharmacology and Psychopharmacology Program
Department of Psychology

8/01-8/02 University of Kentucky, Lexington, KY
Sr. Laboratory Technician with Dr. L.P. Dwoksin
College of Pharmacy

9/99-5/01 University of Massachusetts, Boston, MA
Undergraduate Research and Teaching Assistant with Dr. S.T. Cunningham in Department of Psychology

Scholastic and Professional Honors:

University of Kentucky, Lexington, KY

- Interdepartmental Neuroscience Program Fellowship (2002-2003)
- Graduate School Research Travel Awards (2003-2005, 2007)
- NIDA Predoctoral Research Training Grant: T32 DA07304 (Behavioral Science; 2004-2006)
- The College on Problems of Drug Dependence Travel Award (June 2005)
- Research Challenge Trust Fellowship Travel Award (September 2005)
- NIDA Predoctoral Research Training Grant: T32 DA016176 **(**Biochemistry; 2006-2008)
- Behavioral Neuroscience and Psychopharmacology Graduate Student Achievement Award (2006, 2008)

University of Massachusetts, Boston, MA

Departmental Honors in Psychology
Dean's List: Fall 1998- Spring 2001
Honor Societies: Psi Chi, Golden Key
Undergraduate Research Fund Award (1999-2001)
Associate Provost and Honors Department 1/2000, 1/2001 and 6/2001

Publications:

Dwoskin LP, Pivavarchyk M, Joyce BM, **Neugebauer NM**, Zheng G, Zhang Z, Bardo MT and Crooks PA (in press). Targeting reward-relevant nicotinic receptors in the discovery of novel pharmacotherapeutic agents to treat tobacco dependence. In R. A. Bevins and A. R. Caggiula (Eds.), The motivational impact of nicotine and its role in tobacco use.

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Nichole Marie Neugebauer

07/16/2008