The Dissertation Committee for Allison Anne Feduccia Certifies that this is the approved version of the following dissertation:

EFFECTS OF AUDITORY AND THERMAL STIMULI ON 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA)-INDUCED NEUROCHEMICAL AND BEHAVIORAL RESPONSES

Committee:

Christine L. Duvauchelle, Supervisor

Andrea C. Gore

Edward M. Mills

Rueben A. Gonzales

Timothy Schallert

EFFECTS OF AUDITORY AND THERMAL STIMULI ON 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA)-INDUCED NEUROCHEMICAL AND BEHAVIORAL RESPONSES

by

Allison Anne Feduccia, B.S.

Dissertation

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

The University of Texas at Austin

December 2009

Dedication

This dissertation is dedicated to my parents (Mary and Michael), for encouraging me to set high goals for myself and teaching me "the magic of believing," my siblings (Ashley, Michael, and brother-in-law Michael), for their laughter and good cheer, to my love (Taylor), for his energizing spirit and positive words, and to my best friend (Zydeco), for his unwavering devotion. I am forever grateful for all of their ongoing support, understanding, and unconditional love.

Acknowledgements

I acknowledge,

… My supervisor, Dr. Christine Duvauchelle, for her guidance, expertise, and support throughout my graduate training

… My committee (Dr. Andrea Gore, Dr. Edward Mills, Dr. Rueben Gonzales, and Dr. Timothy Schallert) for their valuable suggestions and help with my dissertation

… Previous graduate students (Dr. Aiko Ikegami, Dr. Elena Reveron, and Dr. Mano D'Souza) for their patience in spending countless hours training me in the lab

… Current graduate student, Esther Maier, and lab technician, Leah McAleer, for all their help and support

… Undergraduate students, for all their time and hard work in the lab

… Waggoner Center for Alcohol and Addiction Research for their financial support by awarding me the Fred M. Jones and Homer L. Bruce Endowed Graduate Fellowship (2006-2009)

EFFECTS OF AUDITORY AND THERMAL STIMULI ON 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA)-INDUCED NEUROCHEMICAL AND BEHAVIORAL RESPONSES

Publication No._____

Allison Anne Feduccia, Ph.D.

The University of Texas at Austin, 2009

Supervisor: Christine L. Duvauchelle

The amphetamine derivative, 3,4-methylenedioxymethamphetamine (MDMA), is a popular drug often taken by young adults at dance clubs or rave parties. Laser light shows, fast-paced electronic music, and hot crowded dance floors are characteristic of these events, and Ecstasy users report that the acute effects of the drug are potentiated by these stimulatory conditions. However, it remains largely unknown how environmental stimuli impact the neurochemical and physiological effects of MDMA. The aim of the first study presented in this dissertation was to investigate how auditory stimuli (music, white noise, and no additional sound) influence MDMA conditioned place preference (CPP), self-administration, and nucleus accumbens (NAcc) dopamine (DA) and serotonin (5-HT) responses. Findings revealed a significant CPP for animals exposed to white noise during MDMA conditioning trials. After self-administration of MDMA (1.5 mg/kg), NAcc DA and 5-HT were highest in rats exposed to music during the test session. The second study aimed to investigate the effects of ambient temperature (23° or 32°C) on long-term MDMA self-administration and neurochemical responses. Results indicated no difference in self-administration or locomotor activity rates for the high versus room temperature groups across sessions. However, MDMA (3.0 mg/kg) administered in high ambient temperature resulted in significantly greater NAcc serotonin release compared to when taken at room temperature, but no differences in dopamine response was determined between the two conditions. Overall, these results indicate that auditory and thermal stimuli can effect MDMA-induced behavioral and neurochemical responses. The last aim tested a novel apparatus and method for use in animal models of drug reinforcement. By combining traditional CPP and self-administration procedures, this approach provided more informative data and circumvented some inherent

drawbacks of each method alone. In addition to confirming the ability to produce drug conditioned place preferences after short- and long-term experiments, the long-term version of the procedure revealed a significant positive relationship between lever response rate and CPP magnitude. Therefore, this experimental design can be used to identify subgroups of rats that may vary in sensitivity to drug motivational effects. Further study of these populations may be useful in the development of behavioral and pharmacological therapies for drug addiction.

Table of Contents

List of Tables

List of Figures

List of Illustrations

CHAPTER 1: BACKGROUND AND SPECIFIC AIMS

1-1. MDMA HISTORY

MDMA was first synthesized by a German scientist, Dr. Anton Kollisch, in 1912 and patented by the pharmaceutical company, Merck, on May 16, 1914 in Germany. At this time, the significance of the discovery was not yet known and MDMA was patented simply as a precursor of a novel chemical pathway for the clotting agent hydrastinine (Bernschneider-Reif et al., 2006). In the 1950s, the drug was researched briefly by the US military in toxicology experiments on five different animal species. However, data from these studies remained unpublished until 1973 (Hardman et al., 1973).

In 1965, Alexander ("Sasha") Shulgin, a chemist living in California, rediscovered and synthesized MDMA. A few years later he began self-testing the drug, incrementally increasing the dose, until he recognized the drug's strong psychoactive effects. After researching the drug on himself and peers, he introduced the drug to his psychologist friend, Leo Zeff. Dr. Zeff began using the drug in psychotherapy and it wasn't long until several psychiatrists picked up on MDMA's ability to help patients overcome emotional barriers and enhance communication during clinical sessions (Bernschneider-Reif et al., 2006).

In the early 1980s, the drug gained popularity in nightclubs and amongst rave cultures leading to its classification as a Schedule 1 controlled substance in the United States on May 31, 1985. Ecstasy continued to gain popularity around the world and became an integral part of the electronic music movement and "rave party scene." This movement

began in Europe in the mid to late 1980s with the emergence of electronic music in clubs, warehouses, and underground parties. The rave-party trend caught on quickly and before long a subculture grew out this movement. Raves consisted of all night dance parties that featured fast-paced techno music and laser light shows. Club drugs, such as MDMA, LSD, cocaine, amphetamines, and ketamine, were widely used among people who attended these parties. Although there has been a decline in raves since the early 2000s due to government regulations, the trend is restarting and gaining momentum in a broad range of populations and settings around the globe.

Since MDMA was placed on the list of Schedule 1 Controlled Substances in 1985, research on the medicinal value of the drug was largely halted because schedule 1 substances are defined as having no currently accepted medical use and have the potential to be highly abused. In 2002, a publication by George Ricaurte in *Science* was highly publicized because it showed recreational doses of MDMA to cause severe neurotoxicity in primates (Ricaurte et al., 2002). The paper was later retracted with the explanation that there was a mix-up with the drug labels, and methamphetamine was used in these experiments, not Ecstasy (Ricaurte et al., 2003). However, the retraction remained unknown to most of the general public and people were left with the notion that small doses of MDMA are highly toxic. It has been speculated that the "drug mix-up" was politically driven and Ricaurte's work should be carefully reexamined (Philipkoski, 2004).

In recent years, new research studies began to evaluate MDMA's potential medicinal uses in psychotherapy for treating posttraumatic stress disorder (PTSD) and severe anxiety. PTSD is a disorder that occurs after a traumatic event and causes the person to

experience severe, persistent anxiety and psychological trauma. Common symptoms associated with the disorder include flashbacks, nightmares, increased arousal, and avoidance of things associated with the traumatic event. MDMA-assisted psychotherapy may be an effective treatment for PTSD because of its ability to reduce the fear response and allow patients to express and experience strong emotions of fear, anger, and grief, which is a necessary step in the therapeutic process. The first peer-reviewed article regarding these studies was published on September 30, 2008 in the *Journal of Psychoactive Drugs* and offered positive outcomes for MDMA-assisted psychotherapy (Bouso et al., 2008). The Multidisciplinary Association for Psychedelic Studies (MAPS) is currently sponsoring further research in several countries around the world, including the U.S.A, Switzerland, Israel, Canada, Jordon, and Spain.

1-2. HUMAN EFFECTS

MDMA is classified under a new class of pharmacological agents called entactogens due to its ability to enhance feelings of emotional closeness and connectedness to others, empathy, love, and insightfulness. Subjective reports state an overall euphoric feeling with heightened senses – especially the sense of touch. MDMA can be ingested as a pure white crystalline solid or more commonly, pressed pills that are often not pure MDMA but a mix of other drugs. Common street names for MDMA include: "Adam," "Ecstasy," "X-TC," "love drug," "X," "molly."

Recreational use is most popular among teenagers and young adults, especially those who attend raves or all night dance parties. Raves and clubs offer environments that are rich in sensory stimulation, such as loud electronic dance music and flashing lights that may

enhance the positive and subjective effects of MDMA. Indeed, it is estimated that 80-95% of people who attend raves use Ecstasy, while 5-15% of young people in general report using the drug (Parrott, 2004). Therefore, the distinctive elements of the drug-taking environment could play a role in both the primary reinforcing effects of the drug as well as the negative consequences associated with MDMA.

Its widespread use is a cause for concern since research in recent years has shown long-term neurotoxic and neurochemical changes in animal brains (Baumann et al., 2006; Glennon and Higgs, 1992; Quinton and Yamamoto, 2006). Human subjects report a higher euphoric state when taking the drug in clubs or raves, as compared to people who take the drug in a less stimulating environment (Hermle et al., 1993; Scholey et al., 2004). However, Ecstasy users can become dehydrated and have a greater chance of suffering more adverse medical problems such as rhabdomyolysis, intravascular coagulation, and acute renal failure when using the drug in such settings (Green et al., 1995; Henry et al., 1992; Parrott et al., 2006). Human Ecstasy users can experience a range of behavioral and cognitive problems associated with taking the drug, including deficits in learning and memory performance (Bolla et al., 1998; Morgan, 2000; Parrott, 2002).

1-3. MECHANISM OF ACTION

The amphetamine derivative, 3,4-methylenedioxymethamphetamine (MDMA), is the main component of the popular recreational drug, Ecstasy. MDMA increases extracellular monoamine concentrations by a wide range of pharmacological actions, including increasing synthesis, carrier-mediated exchange, reuptake and metabolism

inhibition (Cole and Sumnall, 2003; Gough et al., 1991). MDMA induces a rapid release of pre-synaptic 5-HT, DA, NE, and Ach and acts to inhibit the reuptake of monoamines by actions on vesicular and plasma membrane transporter proteins, with the highest affinity for the serotonin transporter (SERT) (Gudelsky and Nash, 1996). Another mechanism by which MDMA increases extracellular monoamine concentrations is by inhibiting MAO_A and MAO_B in all brain regions. MDMA will bind to several different receptors, with the highest affinity for 5-HT_2, α_2-adrenergic, M_1 muscarinic, H_1 receptors, and a lower affinity for M_2 muscarinic, α_1- and β-adrenergic, and 5-HT_1 receptors. MDMA-induced DA increase is mediated by direct or indirect activation of 5-HT_{2A} receptors that are located on GABA neurons. Once these receptors are activated there is a disruption of the negative feedback of GABA, subsequently increasing DA efflux (Cole and Sumnall, 2003; Schmidt et al., 1994). The hypothalamic-pituitary-adrenal axis (HPA) is also activated after MDMA consumption, with increased concentrations of corticosterone, cortisol, and adrenal ascorbic acid (de la Torre et al., 2000). MDMA has been shown to alter the hypothalamic-pituitary-gonadal reproductive axis, by suppressing gonadotropin-releasing hormone (GnRH) mRNA and serum testosterone levels in male rats (Dickerson et al., 2008). Administration of MDMA produces a pronounced release of the hormones prolactin, vasopressin, and oxytocin (Emanuele et al., 2006; Fallon et al., 2002; Forsling et al., 2002; Henry et al., 1998; Nash et al., 1988). The drug also affects the immune system by reducing the number of lymphocytes in circulation and by suppressing T-lymphocyte and immunoglobulin production (Connor, 2004; Connor et al., 1998; House et al., 1995; Pacifici et al., 2001).

1-4. MESOLIMBIC DOPAMINERGIC SYSTEM

The mesolimbic dopaminergic system, also known as the "reward or pleasure pathway," is a pathway in the brain that is activated by rewarding stimuli, such as food, sex, and drugs of abuse (Adinoff, 2004; Volkow et al., 2002). In this pathway, the ventral tegmental area (VTA) connects the limbic areas by projecting to the nucleus accumbens (NAcc) and the prefrontal cortex (PFC). The NAcc, which receives dopaminergic inputs from the VTA, plays an important role in mediating the reinforcing and motivational effects of MDMA and other addictive drugs (Cadoni et al., 2005; Di Chiara et al., 2004; Ritz and Kuhar, 1993). The nucleus accumbens is comprised of two subregions, the core and the shell, which differ in function and morphology (Di Chiara et al., 2004). Several studies describe how the mesolimbic dopaminergic system may become dysregulated after chronic drug use (Badiani and Robinson, 2004; Bardo, 1998; Nestler, 2004).

Illustration 1: Mesolimbic Dopaminergic System

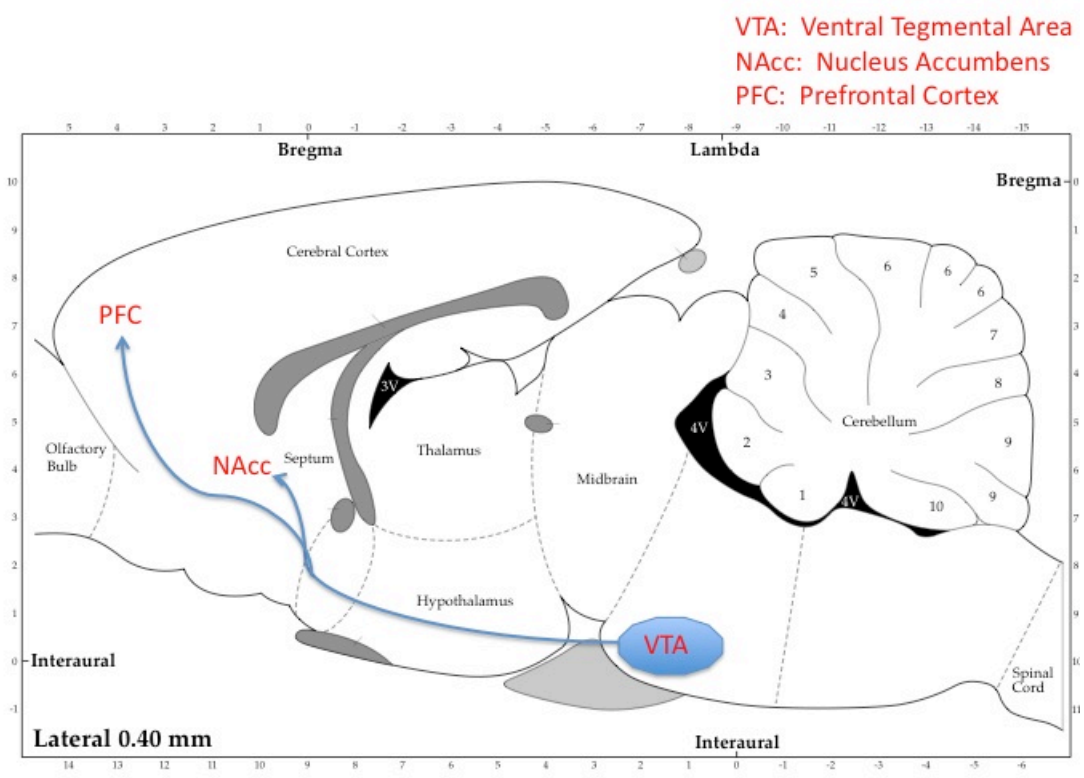


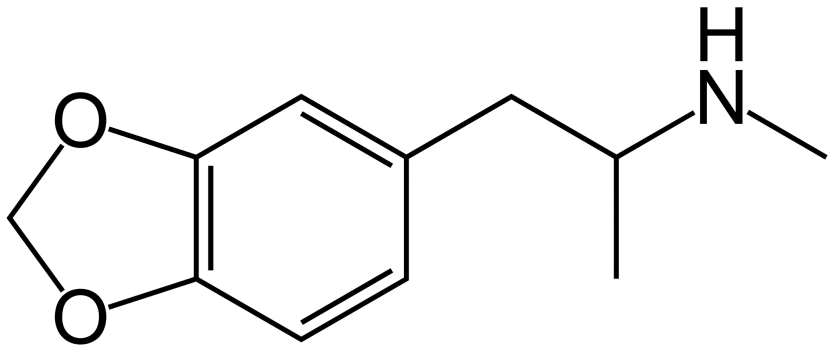
Illustration drawn with the assistance of (Paxinos and Watson, 1997).

1-5. CHEMICAL STRUCTURE

MDMA is a ring-substituted amphetamine derivative, similar in chemical structure to mescaline and methamphetamine. Due to its structure and mechanism of action, MDMA falls under the classification of a phenethylamine, stimulant, psychedelic, and

empathogenic-entactogen. MDMA has R (-) and S (+) optical isomers. The two main metabolic products of MDMA are 3,4-methylenedioxyamphetamine (MDA) and 3,4-dihydroxymethamphetamine (DHMA), which are metabolized further via cathechol-*O*-methyl transferase mediated reactions to 4-hydroxy-3-methoxymethamphetamine (HMMA) and 4-hydroxy-3-methoxyamphetamine (HMA).

Illustration 2: MDMA Chemical Structure



1-6. THERMOREGULATION

Thermoregulation is accomplished by several systems that involve both behavioral and autonomic responses; namely shivering, nonshivering thermogenesis, and cutaneous blood flow (Kanosue et al., 1994b; Morrison, 2001a; Nagashima et al., 2000). The preoptic area (PO) of the rostral part of the hypothalamus receives information from thermorecptors located on the body (surface and core) and the brain to control thermogenic responses that maintain body temperatures. Warm-sensitive neurons in the PO send excitatory efferent signals down through the medial forebrain region to induce

heat loss and inhibitory efferent signals to stimulate heat production (Kanosue et al., 1994a). Neurons in the dorsomedial hypothalamus (DMH) also play an important role in thermoregulatory cutaneous vasoconstriction, shivering, and endocrine responses (Dimicco and Zaretsky, 2007).

Rats' main control of body temperature is through vasodilatation or vasoconstriction of the tails' blood vessels. The rat's tail is well suited for thermoregulation because it is highly vasculated with a large surface to volume ratio and has no fur. In order to cool the body, the tail vessels expand allowing for warm blood to pass through the tail and heat to escape through the surface of the tail. When the blood vessels of the tail constrict, this limits blood flow and conserves heat to increase the body temperature of the rat (Raman et al., 1987; Vanhoutte et al., 2002; Wu et al., 1995). Two regions in the midbrain, the ventrolateral part of the rostral periaqueductal gray and the ventral tegmental area (VTA), receive input from the PO to control blood flow to the tail via postganglionic sympathetic neurons in the intermediolateral cell column. In rats, the tail sympathetic nerve activity is activated by the premotor neurons in the raphe nuclei and not the rostral ventrolateral medulla (RVLM) as seen in other species (McAllen and May, 1994; Morrison, 2001b; Rathner and McAllen, 1999).

Nonshivering thermogenesis has been primarily studied with brown adipose tissue (BAT), which is an important mechanism for heat production especially in small animals. Sympathetic premotor neurons in the rostral raphe nuclei (RnR) project to preganglionic neurons that give direct excitatory cholinergic inputs to interscapular BAT. The activation of uncoupling proteins (UCP), which are found in mitochondrial inner

membranes, provides energy for oxidative phosphorylation and heat production (Mills et al., 2003; Rusyniak et al., 2008; Sprague et al., 2003).

1-7. MDMA AND ELEVATED AMBIENT TEMPERATURE

In animal studies, MDMA has been reported to induce both hypothermia and hyperthermia, depending on several factors such as dose, experience, and ambient temperature (Dafters, 1995; Malberg and Seiden, 1998). The key mechanism of action of MDMA involves the enhancement of extracellular dopamine and to a greater degree, serotonin (Baumann et al., 2008; Koch and Galloway, 1997; Kurling et al., 2008). The induction of hyperthermia by MDMA is likely due to activation of specific receptor subtypes, D1 and 5-HT_{2A}, which leads to the compromise of normal thermoregulatory control (Green et al., 2004a; Mechan et al., 2002; Shioda et al., 2008). Following a dose of MDMA, Mechan et al. did not observe any changes in rats' tail temperatures, even though there was a significant increase in rectal temperatures, indicating that MDMA interferes with normal tail heat loss mechanisms (Mechan et al., 2002). Additionally, nonshivering mechanisms of thermogenesis are important for MDMA-induced hyperthermia. MDMA activates the sympathetic nervous system (SNS), which increases α_1- and β_3-adrenoreceptor modulation of uncoupling protein (UCP3) in skeletal muscles and uncoupling protein (UCP1) in BAT leading to a rise in body temperature (Mills et al., 2003; Rusyniak et al., 2008; Sprague et al., 2003).

In rodents, the magnitude of the hyperthermic response is tightly correlated with symptoms of the serotonin syndrome and MDMA-induced neurotoxicity (Huether et al.,

1997). Equally important, hyperthermia is a prominent symptom of acute MDMA-induced toxicity in humans (Green et al., 2004a). High temperatures may also increase neurotoxicity associated with MDMA (Green et al., 1995; Green et al., 2004b), while low temperatures may help protect against toxicity (Dafters, 1994; Green et al., 2004b; Parrott, 2004). The underlying mechanisms are not clearly understood. The involvement of free radicals is believed to play a role because their formation increased in hyperthermic rats (Colado et al., 1999; Colado et al., 1997; Green et al., 2003).

Cornish *et al*. showed that environmental factors, such as raised temperature, increase intravenous self-administration in animals, thus indicating that heat enhances the rewarding effects of MDMA (Cornish et al., 2003). Furthermore, rats injected with MDMA at high ambient temperatures show a greater enhancement of extracellular 5-HT and DA in the NAcc, compared to rats that receive the drug in lower temperatures (O'Shea et al., 2005).

1-8. ANIMALS AND MUSIC

Insights into the evolution of human music and language have been revealed by comparative studies in animals (Fitch, 2005; 2006). Various animal behaviors are considered to be a parallel of human music making, namely birdsong, whale and seal complex vocalizations, and rat ultrasonic vocalizations. Additionally, African great apes (chimpanzee, bonobos, and gorillas) and monkeys use objects to make structured, periodic sounds (drumming) and other acoustic gestures, such as hand clapping and chest beating (Fitch, 2005). A recent study using the rhesus monkeys, demonstrated that vocal

and nonvocal communication are represented in the temporal lobe and amygdala by overlapping neural networks. Furthermore, drumming communicated information about social status or the emotional state of the drummer to other monkeys (Remedios et al., 2009). Primate drumming behavior may be homologous to human instrumental music, due to its inherent nature and universality in all human cultures.

Other species use acoustic gestures in addition to their vocalizations. For example, rodents drum their paws on the ground in rhythmic fashion (Randall, 2001). Footdrumming is common among desert rodents who have well-adapted ears for hearing low-frequency vibrations in the air or from the ground. A footdrum occurs each time the rat's hind paw hits the ground, and these footdrums are combined into short bursts called footrolls, which are then grouped to make a sequence. In three species of kangaroo rats, each have distinct footdrumming sequences and are believed to communicate territorial ownership, location, and competitive superiority (Randall, 1997; Randall and Lewis, 1997). General auditory constraints used in animal vocalizations are also likely to influence human music, however, from an evolutionary perspective it is unlikely that animal song is analogous with human music. Thus far, animal singing has specific adaptivity for mate attraction or territory defense, while human music is often made for pure enjoyment, beyond the purpose of communication (Hauser and McDermott, 2003).

Similarities in the way animals and humans process complex auditory signals has led to the development of animal models to investigate the effects of music on physiology,

cognition, and emotion (Panksepp and Bernatzky, 2002; Rickard et al., 2005). Animal research allows for more systematic experimental designs and a wider variety of methods (pharmacological, invasive recordings, and lesions) available compared to human research studies. In order for studies from animals to be transferable back to humans, several lines of evidence have demonstrated homologous ways in which animals and humans perceive music. For example, animals can discriminate and respond to basic elements of music, namely variations in tempo, pitch, and loudness (Rickard et al., 2005). One study in anesthetized cats showed that auditory neurons respond to tonal contour (Weinberger, 1988). Another approach investigated perceptual invariance, which "refers to a structural characteristic that is perceived as the same even when transformed." In a discrimination task, European song birds were able to differentiate between various timbers, intensities, rhythms, pitches, pitch patterns, and recognize missing notes in harmonic complexes (Hulse et al., 1984; Page et al., 1989). With regard to pitch processing, unlike humans, the birds performed poorly when the pitches were beyond the range used in training suggesting that birds use absolute pitch processing while humans rely more on relative pitch processing. Furthermore, several species, including rats, dolphins, monkeys show octave equivalence (Blackwell, 1943; Richards et al., 1984; Wright et al., 2000). In sum, animals perceive and discriminate musical sounds in many similar ways to humans indicating similar underlying brain structures for auditory functions (Hauser and McDermott, 2003; Panksepp and Bernatzky, 2002).

Preference tests, approach behaviors, and social activity measures have been used in variety of studies with different species to address the question of whether or not animals have affective responses to music. Peretti and Kippschull demonstrated mice having an enhancement of attraction to other mice, huddling, and sexual behavior when exposed to classical music and a decrease in these behaviors when exposed to rock music (Peretti, 1991). Dogs spent more time resting and less time barking when classical music was played (Wells, 2002) and monkeys showed a decrease in plasma cortisol levels when listening to music (Line, 1991). These studies do not provide conclusive evidence of animals and humans having the same emotional responses to music, but that music is capable of influencing certain animal behaviors.

Other areas of research have investigated the effects of music on cognitive performance, including spatial tasks, learning, and memory. In humans, music has been reported to facilitate performance on a number of cognitive tasks and has positive effects in music therapy for a huge variety of disorders (Abikoff et al., 1996; Avers et al., 2007; Cockerton et al., 1997; Gold, 2007; Wu and Chou, 2008). A number of animal studies have also shown auditory stimuli to enhance learning and memory (Angelucci et al., 2007b; Prior, 2006). There are several theories discussing the underlying mechanisms of music's effects on cognitive performance, most notably the arousal hypothesis. Music, particularly the temporal components, has been shown to increase arousal levels via adrenal stress hormones and neurotransmitters (Britton et al., 1992; Rickard, 2009; Rickard et al., 2005; Sutoo and Akiyama, 2004; Toukhsati et al., 2005). In humans and

animals, enhanced arousal can improve cognitive performance and memory, which could partially explain the effects of music on cognition (Bianchin et al., 1999; Crowe et al., 1990; Rickard et al., 2005). Further evidence for the arousal hypothesis, comes from a recent study by Fang et al. that demonstrated brain music decreases sleep and increases arousal levels in rats (Fang et al., 2009). Additionally, music may also be providing an enriched environment, which can facilitate neural plasticity (Angelucci et al., 2007a; Angelucci et al., 2007b; Cai et al., 2009). In sum, animal models significantly contribute to the evolutionary study of music and research involving the physiological and cognitive effects of music. As with many other animal models used in neuroscience, information gathered from these studies is not 100% analogous to humans but still gives valuable information that is transferable back to human research.

1-9. AUDITORY STIMULI AND AMPHETAMINE DERIVATIVES

Little is known about the reinforcing properties of music on the Ecstasy experience. However, gathering evidence is beginning to show the neurotoxic effects of amphetamines and amphetamine derivatives may be increased by loud noise (Gesi et al., 2004; Scholey et al., 2004). Iannone *et al*. reported a marked increase in electrocortical activity of the telencephalic cortex in rats treated with MDMA (3 mg/kg and 6 mg/kg) and acoustic stimulation (white noise; 95 Db) compared to rats that received MDMA in the absence of additional environmental sound (Iannone et al., 2006). In another study, mice given methamphetamine (75 mg/kg) while exposed to loud music (95 Db) showed a change in a number of behavioral parameters and experienced more seizures than mice

given the same dose of methamphetamine in silence (ambient noise, 55 Db) or in the presence white noise (95 Db) (Morton et al., 2001). Cagiano *et al*. recently published a paper demonstrating low-doses of MDMA (3 mg/kg) in combination with loud music will increase the number of male rats showing sexual activity compared to rats administered the drug without music (Cagiano et al., 2008).

Evidence also suggests that music influences the dopaminergic mesolimbic pathway in the brain, the same pathway that is activated by drugs of abuse and natural rewards, such as food and sex. Music improves dopaminergic neurotransmission and increases neostriatal DA concentrations and serum calcium levels in spontaneously hypertensive rats (SHR). Systolic blood pressure was significantly decreased after these rats were exposed to music due to increased DA activity on D_2 receptors (Sutoo and Akiyama, 2004). A human fMRI study showed the mesolimbic dopaminergic system, including the NAcc and VTA, is highly activated and tightly connected during music processing. The hypothalamus, an important area for the physiological and autonomic response to emotional stimuli (Blood and Zatorre, 2001; Menon and Levitin, 2005), was also activated when subjects listened to pleasant music. By using both functional (correlation of activations between regions) and effective connectivity (interactions of brain regions by anatomical connections) analyses, researchers concluded that increases in NAcc and VTA dopamine levels may underlie the rewarding and reinforcing aspects of listening to music (Menon and Levitin, 2005). Taken together, these findings indicate that music and MDMA may work on common neural networks involving reward and arousal and most likely influence each other. Since Ecstasy is often taken in environments rich in sensory stimulation, it is relevant to investigate the role music plays in enhancing the MDMA experience. A pertinent question to address is whether music offers additional

arousal, which may potentially increase the toxicity of the drugs, and whether sound level and intensity cause any additional altered effects.

1-10. MDMA NEUROTOXICITY

MDMA has been shown to change many markers of serotonergic nerve terminals, including depletions of tissue 5-HT levels and SERT function (Baumann et al., 2006; Glennon and Higgs, 1992; Quinton and Yamamoto, 2006; Schmidt et al., 1987; Stone et al., 1987b). Anatomical studies reveal that the 5-HT cell bodies remain intact, but axonal projections into other brain areas are significantly reduced (Fischer et al., 1995; O'Hearn et al., 1988). The main metabolite of MDMA, 5-hydroxyindoleacetic acid (5-HIAA), and the rate-limiting enzyme in 5-HT synthesis, tryptophan hydroxylase (TPH), are reduced after single or repetitive doses of MDMA (Schmidt and Taylor, 1987; Schmidt et al., 1986; Stone et al., 1987a; Stone et al., 1987b). The neurotoxic effects of MDMA may be caused by the increase of free radical formation and oxidative stress in the brain (Cadet et al., 2001; Colado et al., 1997; Halliwell and Kaur, 1997; Halliwell et al., 1991).

However, the majority of the studies that investigated MDMA neurotoxicity in rats used extremely high dosing regimens of 10 mg/kg or higher (Battaglia et al., 1987; Commins et al., 1987; O'Hearn et al., 1988; Schmidt, 1987; Stone et al., 1987a), which may not be comparable to recreational doses taken by humans (Green et al., 2003). Evidence suggests that a dose of 1-2 mg/kg of MDMA will produce similar pharmacological effects in both rats and humans (Glennon and Higgs, 1992; Gudelsky and Nash, 1996; Kankaanpaa et al., 1998; Oberlender and Nichols, 1988). Therefore, it is important to

investigate whether low doses of MDMA cause any long-term changes of 5-HT neuronal markers, as well as, the role the environment plays in MDMA-induced neurotoxicity.

1-11. MDMA BEHAVIORAL EFFECTS

MDMA dose-dependently enhances locomotor activity, which is mediated by an interaction between dopamine and serotonin (Bankson and Cunningham, 2001; 2002). Evidence for this interaction comes from studies reporting lesions (6-OHDA) of the DA system did not completely abolish MDMA-induced hyperactivity (Gold et al., 1989) and 5-HT_{1B} receptor agonists lead to increased locomotion similar to that seen with MDMA (Rempel et al., 1993). Furthermore, treatment with 5-HT_{2A} receptor antagonist reduced MDMA-stimulated hyperlocomotion, indicating the importance of 5-HT_{2A} receptors in this response (Kehne et al., 1996). Additionally, transgenic mice without SERT or 5-HT_{1B} receptor lack MDMA-induced hyperlocomotion. Baumann *et al*. showed MDMA-stimulated monoamine (DA and 5-HT) release to be positively correlated with locomotor activity and stereotypy behaviors (Baumann et al., 2008). The increase in activity is predominantly seen in the periphery of the testing chamber, where as amphetamines increase activity throughout the entire chamber (Callaway et al., 1990; Gold and Koob, 1989; McCreary et al., 1999). As seen with psychostimulants, repeated low doses of MDMA can elicit locomotor sensitization (Spanos and Yamamoto, 1989). Certain stereotypical behaviors have been associated with the massive release of serotonin, which can be exacerbated by high temperatures, and have come to be known as the serotonin syndrome (Kutscher and Yamamoto, 1979; Piper et al., 2005; Spanos and Yamamoto, 1989). Serotonin syndrome behaviors in rats are described as forepaw treading (lateral side-to-side movement of the forepaws with little motion of the hindlimbs), headweaving

(lateral side-to-side movement of the head with no forward locomotion), low body posture (underbelly nearly touching the floor and the hindlimbs drawn away from the axis of the body) (Hiramatsu et al., 1989; Spanos and Yamamoto, 1989). In humans the symptoms of serotonin syndrome include hyperactivity, mental confusion, agitation, fever, tachycardia, shivering, clonus, myoclonus, ocular oscillations, and tremor (Green et al., 1995; Huether et al., 1997). This severe adverse drug reaction is unusual and often caused by serotonergic drug interactions with MDMA, such as monoamine oxidase inhibitors (MAOIs) and selective serotonin reuptake inhibitors (SSRIs). Medical intervention involves cooling the body and the use of 5-HT_2 and 5-HT_{1A} antagonists (Gillman, 1998; 1999).

1-12. Animal Models of Drug Reinforcement

Animal models of addiction, including place conditioning procedures and operant drug self-administration, are highly reliable and widely used for estimating the abuse potential of drugs in humans (Bardo and Bevins, 2000; Bozarth et al., 1980; Tzschentke, 1998). There have been over 1000 new published reports using place conditioning procedures since 1998, with the number of publications increasing each year (Tzschentke, 2007). Place conditioning procedures are used to measure the subjective, reinforcing or aversive properties of natural rewards and drugs of abuse. The drug's reinforcing or aversive effects act as an unconditioned stimulus (UCS) and after several pairings with neutral environmental cues; the environment becomes a conditioned stimulus (CS) and then alone can elicit approach/avoidance behaviors. Such behaviors can be measured to provide information regarding the reinforcing properties of the drug. The apparatus used

in this paradigm consists of a conditioning box with two distinct environments usually differing in color, floor texture, lighting, or odor. After multiple days of conditioning, one compartment becomes associated with the drug, while the other compartment is linked to saline. On the test day, animals in a drug-free state are allowed access to both compartments. A conditioned place preference (CPP) is indicated by an increase in time spent in the drug-paired compartment and a conditioned place aversion (CPA) by a decrease in time spent in the drug-paired compartment.

In rats, most drugs that produce a CPP are also self-administered and vice versa, drugs that don't produce a CPP aren't self-administered. However, there are a few examples where this is not the case: LSD, buspirone, and pentylenetetrazole produce a CPP but is not self-administered, whereas pentobarbital and phencylclidine are self-administered but no CPP is observed (Bardo and Bevins, 2000).

Operant self-administration models are extensively used in pre-clinical drug research on account of being a reliable and valid measure in predicting drug consumption in humans (Fischman and Schuster, 1978; Gardner, 2000; Panlilio and Goldberg, 2007; Schuster and Thompson, 1969). Consequently, these procedures are employed to find strategies that are useful in terminating drug use, treating addiction, and deterring future drug-seeking and relapse in abstinent users. Numerous paradigms and conditions extend from basic operant self-administration to model different aspects of addiction. Namely, these are schedules of reinforcement (simple fixed ratio schedules, progressive-ratio schedules, multiple schedules), route of administration (intravenous, oral, inhalation, intracranial), and speed of drug delivery (Arnold and Roberts, 1997; Meisch, 2001; Roberts et al., 2007).

Operant self-administration and place conditioning procedures are valuable behavioral pharmacological approaches to studying drug addiction and reinforcement (Panlilio and Goldberg, 2007; Tzschentke, 2007). However, both methods have inherent methodological drawbacks. One large drawback of the conditioned place preference procedure/chamber is that the drug is administered non-contingently, which has been shown to change a variety of behavioral and neurochemical aspects as compared to the same drugs that are self-administered (Miguens et al., 2008; Moolten and Kornetsky, 1990; Palamarchouk et al., 2009; Stefanski et al., 2007; Twining et al., 2009). The differences can be attributed to the mode of drug intake and is not an accurate model of the human experience (Collins et al., 1984). Furthermore, CPP does not provide any information regarding changes in motivation to seek drugs or drug dependence. The operant self-administration method also has potential pitfalls. The rates of responding are used to infer reward value but are affected by motor drug effects. The ability to test conditioned place preference in a drug-free state circumvents this problem. Furthermore, self-administration procedures can be time consuming and expensive to conduct. Combining self-administration experiments with place conditioning would offer a more time and cost-effective approach.

1-12. MDMA PLACE CONDITIONING AND SELF-ADMINISTRATION EXPERIMENTS

MDMA causes a dose dependent conditioned place preference in rats, with optimal doses ranging from 2.0–10.0 mg/kg (Bilsky et al., 1990; Marona-Lewicka et al., 1996). However, other non-drug factors may be crucial to the development of the MDMA CPP. Meyer et al. showed that the housing conditions of the animals influenced the ability of

MDMA to produce a significant CPP, with isolated animals showing a more pronounced CPP than group-housed rats (Herzig et al., 2005; Meyer et al., 2002). Although MDMA produces a positive CPP, it has not been shown to be a potent reinforcer in self-administration studies (De La Garza et al., 2006; Ratzenboeck et al., 2001). Laboratory rodents and non-human primates will self-administer MDMA but response-acquisition is more gradual in comparison to other drugs, such as cocaine or methamphetamine (Fantegrossi et al., 2004; Reveron et al., 2006; Schenk et al., 2003).

1-14. COCAINE PLACE CONDITIONING AND SELF-ADMINISTRATION EXPERIMENTS

A large number a studies show the dopaminergic drug, cocaine, induces a conditioned place preference and several factors influence the magnitude of place conditioning, namely: species, dose, route of administration, number and duration of drug-pairings, experimental procedure, sex, and age (Russo et al., 2003; Zakharova et al., 2009a; Zakharova et al., 2009b). Adult female and both male and female periadolescent rats show cocaine CPP at a lower dose than adult male rats (Russo et al., 2003; Zakharova et al., 2009b). A large range of doses can induce CPP, as well as, various routes of administration (i.p., s.c., and i.v.) (Bardo et al., 1999; Sellings et al., 2006; Tzschentke, 2007). A meta-analysis of cocaine CPP in the literature by Bardo *et. al.*, showed no dose-CPP effect relationship meaning there was no significant differences between the strength of CPP produced by a range of cocaine doses (0.1 – 10 mg/kg, i.v., s.c., and i.p. combined) included in the analysis (Bardo et al., 1995). However the authors note, that other studies have shown IP cocaine administration to produce a monotonic ascending dose-effect curve and IV administration to produce an inverted U-shaped dose-effect

curve (Nomikos and Spyraki, 1988). Separate analysis of each route of administration by Bardo *et. al*., also failed to show a dose-effect response for cocaine CPP (Bardo et al., 1995). Other factors (see above in this section) than dose seem to have a greater impact on the magnitude of cocaine-induced CPP.

Cocaine is readily self-administered by several different animal species and a large body of evidence illustrates that the reinforcing effects is dependent on dopamine transmission in the mesolimbic dopaminergic system (Ikegami et al., 2007; Pettit and Justice, 1991; Rocha et al., 1998). Cocaine self-administration rates can be affected by a number of factors: schedule of reinforcement, prior drug experience, dose, injection speed, and drug deprivation (Liu et al., 2005; Roberts et al., 2007; Sellings et al., 2006).

1-15. SUMMARY AND SPECIFIC AIMS

The environmental contexts in which drugs are taken have been shown to modulate drug-induced neuroadaptations (Badiani and Robinson, 2004). Indeed, drug-associated environmental stimuli have the ability to induce drug craving and elicit drug seeking in both animals and humans (Childress et al., 1993; O'Brien et al., 1992). Since MDMA is often used at clubs and raves, which offer environments rich in sensory stimulation, it is important to investigate how these settings influence the Ecstasy experience. Loud music and high ambient temperatures may heighten the subjective effects of the drugs, but may also enhance the deleterious effects of the MDMA. Since environmental factors, such as raised temperature have been shown to increase responding for MDMA (Cornish et al.,

2003), it is important to investigate the role of other contextual components in initiation and maintenance of MDMA self-administration.

The goal of the chapters 2 and 3 is to determine if environmental acoustic stimulation and increased ambient temperatures potentiate the neuropsychobiological effects and reinforcing properties of MDMA. The focus of these chapters will be on behavior parameters that may be altered as a result of taking MDMA under varying conditions, as well as, changes in NAcc monoamines levels measured by *in vivo* microdialysis. These chapters of the dissertation will provide insight into the neurochemical and behavioral changes associated with using MDMA in popular settings, such as dance clubs and raves.

Animal models are useful and valid tools for studying mechanisms of drug reinforcement and are critical for advances in addiction research. Place conditioning and self-administration procedures are used extensively throughout the literature to investigate several facets of drug abuse and addiction including: (a) abuse liability, (b) drug intake, (c) drug seeking, (d) initiation, maintenance, and reinstatement (e) reinforcement and reward, (f) drug-induced tolerance and sensitization and (g) withdrawal and relapse. Although these methods have proven insightful, there are inherent drawbacks associated with both of these procedures. In chapter 4, a novel apparatus and approach combines traditional CPP with self-administration to provide a measure that more accurately models human drug consumption and gives more informative data than either method alone.

Specific Aim 1: To determine the effects of auditory stimuli on MDMA conditioned place preference, self-administration, and NAcc dopamine and serotonin responses (chapter 2).

Specific Aim 2: To determine the effects of thermal stimuli on long-term MDMA self-administration, locomotor activity, core body temperature changes, and NAcc dopamine and serotonin responses (chapter 3).

Specific Aim 3: To test a novel apparatus and approach for use in animal models of drug reinforcement (chapter 4).

CHAPTER 2: AUDITORY STIMULI ENHANCES MDMA-CONDITIONED REWARD AND MDMA-INDUCED NUCLEUS ACCUMBENS DOPAMINE, SEROTONIN AND LOCOMOTOR RESPONSES

2-1. ABSTRACT

MDMA (3,4-methylenedioxymethamphetamine), also known as Ecstasy, is a popular drug often taken in environments rich in audio and visual stimulation, such as clubs and dance parties. The present experiments were conducted to test the notion that auditory stimulation influences the rewarding effects of MDMA. In Experiment 1, a conditioned place preference (CPP) procedure was conducted in which rats received MDMA (1.5 mg/kg, s.c.) in a distinctive environment accompanied by music (65-75 dB), white noise (70 db), or no added sound. Animals were pre-treated with saline on alternating days in an alternate environment. Results revealed CPP in animals exposed to white noise during MDMA trials. For Experiment 2, rats from Experiment 1 had access to operant levers that delivered intravenous MDMA (0.5 mg/kg/inj) or saline (0.1 ml) on alternate days in the presence or absence of the same types of auditory stimuli as previously experienced. After three each of MDMA and non-reinforced (saline) sessions, animals were tested for NAcc DA and 5-HT responses to MDMA (1.5 mg/kg) or saline under the same stimulus conditions. Findings revealed that NAcc DA and 5-HT increased after an MDMA injection, and both DA and 5-HT were significantly highest in animals exposed to music during the test session. These results indicate that paired sensorial stimuli can engage the same systems activated during drug use and enhance neurochemical and behavioral responses to MDMA administration.

2-2. INTRODUCTION

MDMA (3,4-methylenedioxymethamphetamine), also known as Ecstasy, is a popular drug often taken at clubs and raves, particularly by adolescents and young adults. Raves and clubs offer environments that are rich in sensory stimulation, such as loud electronic dance music and flashing lights that may enhance the positive and subjective effects of MDMA. Indeed, it is estimated that 80-95% of people who attend raves use Ecstasy, while 5-15% of young people in general report using the drug (Parrott, 2004). Therefore, the distinctive elements of the drug-taking environment could play a role in both the primary reinforcing effects of the drug as well as the negative consequences associated with MDMA.

Although MDMA use occurs most frequently in environments rich in sensory stimulation, little is known about the reinforcing properties of music on the Ecstasy experience. Evidence suggests that music influences the dopaminergic mesolimbic pathway in the brain, the same pathway that is activated by drugs of abuse and natural rewards, such as food and sex. For instance, human fMRI studies show the mesolimbic dopaminergic system, including the NAcc and VTA, are highly activated during music processing (Blood and Zatorre, 2001; Menon and Levitin, 2005), and music increases dopaminergic neurotransmission and neostriatal DA concentrations in spontaneously hypertensive rats (SHR) (Sutoo and Akiyama, 2004). These findings indicate that music activates common neural networks involved in reinforcement and arousal, raising the possibility that MDMA and music may be mutually influential on reward pathways.

Animal models of addiction, including conditioned place preference (CPP) and drug self-administration are useful in predicting or confirming drug candidates with abuse potential in humans. However, even though MDMA is a very popular drug in party-like atmospheres, response rates and speed of acquisition for MDMA self-administration are low (Reveron et al., 2006; Schenk et al., 2003), especially when compared to cocaine (Lile et al., 2005). Since human MDMA use patterns are typically intermittent, it is possible that the reinforcing properties of MDMA are difficult to assess using standard animal models of addiction. Another possibility is that MDMA administered in the absence of additional sensory stimuli (e.g., music, noise, lights) may not truly reflect the subjective rewarding qualities of MDMA intake within a sensory-rich environment.

In the experiments presented here, rats were exposed to intermittent, low dosages of MDMA to test the hypothesis that auditory stimuli present in environments paired with MDMA influence behavioral and neurochemical responses to the drug. For the first stage of the experiment, a 6-day CPP procedure was performed in which 3 days of MDMA treatment (1.5 mg/kg) alternated with 3 days of saline injections (0.1 ml). During MDMA sessions, rats were exposed to music (65-75 dB), white noise (70 dB) or no additional sound (ambient noise approx 52 dB). CPP was assessed on Day 7 in the absence of MDMA and auditory stimuli. In Experiment 2, a sub-group of the same rats were trained to lever press for food reinforcement. The animals subsequently underwent intravenous catheterization and stereotaxic surgery to enable drug self-administration and *in vivo* microdialysis testing. During cue conditioning/ operant sessions (which commenced 3 weeks after the CPP test), rats were allowed access to levers that, when activated, delivered MDMA (0.5 mg/kg/inj) or saline (0.1 ml) over 6 alternating days (3 MDMA and 3 saline sessions) in the presence of visual and auditory stimuli similar to the

CPP conditions. On Day 7 of this experimental phase, rats were tested for NAcc DA and 5-HT responses to a self-administered MDMA injection or to a non-reinforced operant response in the presence or absence of auditory stimuli.

2-3. MATERIALS AND METHODS

2-3-1. Animals

100 adult male Sprague-Dawley rats (200-250 g at the start of the experiment), obtained from the University of Texas Animal Resource Center, were used in this study. Two weeks prior to the experiment, animals were handled daily. Rats were group housed in plastic cages; after surgery they were housed individually. They had free access to food and water and were on a 12:12 reverse light dark cycle. Animals were kept in accordance with the Guide for the Care and Use of Laboratory Animals (U.S. Public Health Service, National Institute of Health) and the Institutional Animal Care and Use Committee (IACUC) at the University of Texas at Austin.

2-3-2. Drug and Sensory Stimuli (Cues)

MDMA

(+/-) 3,4-methylenedioxymethamphetamine HCl used in this experiment was provided through the NIDA Drug Inventory Supply and Control Program (RTI International, Research Triangle Park, NC). The drug was dissolved in isotonic saline solution (0.9 %) for intravenous and subcutaneous injections.

Sensory Stimuli

Auditory stimuli consisted of music (The Very Best Euphoric House Breakdown; Telstar Records, UK) similar to the genre of music that would be played in a club or rave, and white noise (Pure White Noise, Tallahassee, FL). Two speakers produced sound at 65-75 dB, measured by a sound pressure level meter (Galaxy Audio, Wichita, KS). Visual stimuli consisted of all black or all white walls in the place conditioning apparatus and operant chambers. During operant trials, olfactory stimuli (cinnamon or rose oil-based scents) were also paired with visual and auditory cues.

2-3-3. Apparatus

Place Preference

The place preference apparatus used consisted of two compartments (26.6 x 27.6 x 31 cm) separated by a removable piece of Plexiglas. One compartment had white walls and chicken wire over the white floor. The other compartment had black walls and a black Plexiglas floor.

Operant Conditioning Chambers

Operant chambers (28 x 22 x 21 cm) located within sound-attenuating compartments (Med Associates, St. Albans, VT) were used for food training, self-administration and *in vivo* microdialysis sessions. Chambers had a single lever on the right wall and a stimulus light above the lever. Three sets of photocells were located on the lower front and back walls of the chamber. Breakages in the photocell beams were recorded as locomotor

activity units. Lever presses and locomotor activity were recorded during each session using a Med Pentium 100 MHZ computer equipped with Med-PC software.

2-3-4. Conditioning and Training

Experiment 1: Conditioned Place Preference

Treatment Groups.

In three experimental groups, animals were pretreated with MDMA (1.5 mg/kg) 5 minutes prior to placement into one compartment of the apparatus. After placement within the compartment, animals were exposed to music, white noise or no additional sound. On alternate days in the alternate compartment, saline pretreatment was received 5 minutes prior to placement and animals were not exposed to additional sound stimuli during the session. Three control groups were pretreated with saline and were placed in the compartment and exposed to music, white noise or no additional sound. On alternate days in the other compartment, these groups received saline pretreatment, but no added auditory stimulation (See CPP Groups, Table 1).

CPP Procedure.

Animals were allowed access to both compartments of the CPP apparatus to determine the Baseline preference. For this procedure, rats habituated to the apparatus for the first five minutes and were then timed for the duration spent in each compartment (e.g., black side versus white side) over the following 15 min period. Conditioning sessions commenced the next day. On Conditioning Days 1, 3, and 5, animals were injected with MDMA (1.5 mg/kg, s.c.) and confined to one side of the apparatus for 40 min. On alternate days, an equal volume of saline was injected and rats were confined to the alternate compartment for 40 min. Control groups received saline prior to placement in

both sides of the apparatus for all 6 days. 24 hrs after the last conditioning session, animals were tested for place preference. In the Test session, rats did not receive an injection, but were directly placed within the apparatus and allowed access to both compartments for 20 minutes. As with the Baseline preference test, after a 5 min habituation period, the time spent in each compartment was measured for a total of 15 minutes.

Experiment 2: Cue Conditioning/Operant Sessions and In vivo Microdialysis

Food training.

After completing the CPP experiment, a subgroup of animals (with equal MDMA intake experience) was food restricted (≈ 6 g of standard rat chow per day, adjusted as needed to maintain body weight) and trained to lever press for food on a FR1 schedule of reinforcement. Each lever response resulted in dispensing one sucrose pellet (45 mg; P.J. Noyes, Lancaster, NH). After the lever press response for food was acquired (approx 3 days), 10-min. food-reinforced operant sessions (FR1) were conducted for the next 6 days without food restriction.

Surgical Procedure.

To enable voluntary intake of MDMA and in vivo microdialysis testing, jugular catheterization and stereotaxic surgery to implant a guide cannula was performed. Rats were anesthetized with pentobarbital sodium (Nembutal®; 50 mg/kg, i.p.) and chloral hydrate (80 mg/kg, i.p.) as needed to prolong the anesthesia. Rats received atropine sulfate (250 ug/rat, s.c.) for prevention of respiratory secretions, and the anti-inflammatory agent, Rimadyl (5 mg/kg, s.c.), post-surgically to alleviate pain. Catheters

were constructed from 8.5 mm Silastic tubing (0.64 mm o.d.) with one end connected to a cannula endpiece (Plastics One, Roanoke, VA). The catheter was inserted into the right jugular vein and the cannula endpiece passed subcutaneously to an incision on the head. Animals were stereotaxically implanted with a unilateral guide cannula (21 g; Plastic One, Roanoke, VA) above the NAcc (AP: + 0.2 mm, ML: +/- 0.12 mm; DV: -2.5 mm). NAcc microdialysis probes extended 6.25 mm past the ends of the cannula. The catheter and cannula was anchored to the top of the head with four stainless steal screws and dental acrylic cement. For the first week after surgery, catheters were flushed daily with 0.1 m of 0.9% saline that contained U/ml heparin and 67.0 mg/ml Timentin. Animals continued receiving the same solution daily without the Timentin component through the duration of the experiment to maintain catheter patency.

Treatment Groups.

Rats were assigned to one of five groups in which different auditory, visual and olfactory cues were present while rats had the opportunity to press a lever resulting in intravenous MDMA or saline injections. Group assignments for each rat were in correspondence with their previous CPP treatment conditions, except that olfactory stimuli were added as an additional cue during operant sessions. Three groups were exposed to music (MDMA + Music), white noise (MDMA + Noise) or no additional sound (MDMA Alone) during MDMA self-administration sessions, and no additional sound during non-reinforced sessions. The other groups were exposed to MDMA and no additional sound during MDMA sessions and either music (Music Alone) or white noise (Noise Alone) during non-reinforced operant sessions.

Cue Conditioning/Operant Sessions.

Conditioning sessions commenced after a seven-day surgery recovery period. During these sessions, MDMA (0.5 mg/kg per infusion) or saline (0.1 ml/inj) was available for self-administration. On Days 1, 3, and 5, rats had access to MDMA, and on Days 2, 4, and 6, lever responses resulted in saline infusions. For the first half hour of the session, the operant chamber remained dark and the lever was retracted. After 30 minutes, the house light came on and the cues (visual, olfactory and auditory) were presented. 5 seconds later, the lever became available for operant responding for a total of 30 minutes.

Microdialysis Procedures

In Vitro Recovery Calibration.

Microdialysis probes were constructed as previously described (Duvauchelle et al., 2000) with an active membrane length of 2.5 mm at the probe tip. Prior to probe recovery, all probes were flushed with nanopure water. Recovery values for each probe were calculated by comparing the peak heights of samples to those from a standard as previously described (Ikegami et al., 2007). The mean (± SEM) recovery of probes used in the experiment was 12.28 ± 0.44% for DA and 12.90 ± 0.50% for 5-HT.

Microdialysis probe implantation.

Within 12 hrs after the 6th self-administration session, animals were briefly anesthetized with isoflurane and implanted with a microdialysis probe through the previously implanted guide cannula. Each microdialysis probe was connected to a 1.0 ml gastight Hamilton 1000 series syringe mounted on a syringe pump (Razel®, Model A), and freshly prepared Ringer's solution was pumped through the probe. Animals implanted with the probe remained in a holding chamber overnight with the syringe pump speed set

at 0.2 μl/min. Bedding, food, and water were available in the holding chamber. One hour prior to the test session, the pump speed was increased to 1.6 μl/min.

Test Session for NAcc DA and 5-HT Responses

24 hrs after the last self-administration session, rats were tested for their NAcc DA and 5-HT responses in one of six different Test conditions. Animals that had self-administered MDMA in the presence of music or white noise were tested for DA and 5-HT responses to MDMA under the same conditions (MDMA + Music and MDMA + Noise). Animals that were exposed to music or white noise during access to the non-reinforced lever (saline) received saline under the same conditions at test (Music Alone and Noise Alone). For animals that self-administered MDMA in the absence of additional auditory stimuli, some received MDMA at test (MDMA Alone) and the others received saline (Saline Alone). For the test session, animals were placed in the operant chamber with the lever retracted for the first 30 min (Baseline), as previously described for self-administration sessions. After 30 min., the house light illuminated, the lever extended into the chamber and the sensory cues associated with either MDMA or saline operant sessions were introduced into the chamber. Animals were allowed to respond once on the lever and received either a single self-administered injection of MDMA (1.5 mg/kg) or saline (0.1 ml) infused over a 6-sec interval. The lever was then retracted for the remainder of the session. *In vivo* microdialysis samples were collected at 10 min intervals across the entire test session, comprising 3 10-min Baseline and 3 10-min Test (e.g., post-MDMA or saline injection) samples. Locomotor activity units (photobeam breakages) were assessed in correspondence with dialysis sampling.

Analysis of DA and 5-HT

High Performance Liquid Chromatography with electrochemical detection (HPLC-EC; Shizeido Capcell C-18 narrow bore column, ESA model 5200A Coulochem III Detector, Model 5041 cell and a Model 5020 Guard Cell; ESA, Inc., Chelmsford, MA) was used to determine DA and 5-HT levels. An ESA model 420 dual pistons HPLC pump pushed 0.2 ml/min Mobile Phase through the system. The mobile phase composition was as follows: sodium dihydrogen phosphate (75 mM), citric acid (4.76 mM), SDS 1 g/l, EDTA (0.5 mM), MeOH 8% and acetonitrile 11% (v/v), pH 5.6. The analytical cell potential was set at 100 mV (oxidation), guard cell potential at 400 mV, and the pump speed at 0.2 ml/min. The detection limit of DA was 0.1 nM with a signal to noise ratio of 3:1. The detection limit of 5-HT was 0.12 nM with a signal to noise ratio of 3:1. The amount of DA and 5-HT within each sample was determined by comparison with standards prepared and analyzed on the day of sample analysis. Prior to correcting for probe recovery, mean (± SEM) basal NAcc DA and 5-HT concentrations (first baseline sample) for all animals included in the dialysis portion of the experiment was calculated at 0.54 ± 0.06 nM and 0.21 ± 0.05 nM, respectively (n=50). Data were collected and analyzed using an ESA Model 500 Data station.

2-3-5. Histology

Animals were sacrificed at the end of the experiment and brains were stored in 10% formaldehyde/30% sucrose solution. Dialysis data obtained only from animals with probe locations confirmed to be in the NAcc from 48 μ coronal sections (see Fig 1).

2-3-6. Statistical Analysis

Conditioned Place Preference

Difference scores at baseline and test were used to depict the amount of time spent oneach side of the place preference apparatus. For rats receiving MDMA during conditioning, difference scores were calculated by subtracting the number of seconds spent on the saline-paired side from time spent on the drug-paired side. For the control groups, difference scores were calculated by subtracting the saline-paired side from the saline + sound-paired side. Therefore, significant positive differences between Baseline and Test scores reflect CPP. A one-way analysis of variance (ANOVA) was performed across all groups' baseline difference scores. CPP's were determined by comparing MDMA-receiving groups with the Saline Control using a 2-way analysis of variance with repeated measures (Group X Time [Baseline, Test]) on the difference scores. Two additional control groups were also compared to the Saline Control in a separate 2-way ANOVA. Post hoc analyses (Fisher's Least Significant Difference) were utilized to detect significant group differences (at least $p < 0.05$) when main effects were detected in the initial analyses.

Operant Sessions

The number of lever responses elicited during the three MDMA- and non-reinforced operant sessions was compared across groups using a three-way ANOVA (Group X Treatment Level [MDMA and Non-reinforcement] X Days).

Microdialysis Test Session

To compare the magnitude of NAcc DA and 5-HT responses between animals receiving different sensory stimuli during self-administration and test sessions, data from animals receiving MDMA and those receiving saline during the test session were analyzed using two-way ANOVA with repeated measures (Group X Time [3 Baseline and 3 Test intervals). DA and 5-HT concentrations in nM was corrected according to probe recovery rates and converted to percent of baseline for data analyses (overall baseline averaged from within-subject means of three baseline measurements). Locomotor activity, assessed by photobeam breakages within the operant chamber during the test session, was analyzed in the same manner using two-way repeated measures ANOVAs. Post hoc analyses (Fishers LSD) for dialysis and locomotor activity data were used to detect specific group or time differences (e.g., at least $p < 0.05$) when main and/or interaction effects were indicated by overall analyses.

2-4. RESULTS

2-4-1. Experiment 1: Conditioned Place Preference

A one-way ANOVA performed on baseline difference scores of all experimental groups showed no significant differences [$F(5,99) = 0.506$, n.s.]. A two-way repeated measures ANOVA (Group x Time) comparing the MDMA-conditioned animals with the Saline Alone control revealed significant Time [$F(1,70) = 28.92$, $p < 0.0001$] and Group effects [$F(3,70) = 3.22$, $p < 0.05$], but no significant Interaction [$F(3,70) = 0.814$, n.s.]. Post hoc analyses showed that at test, only the MDMA + Noise group had a significantly higher score than the Saline Alone group (see Fig 2A). A two-way repeated measures ANOVA

(Group x Time) performed on the three control conditions revealed no significant Group, Time or Group X Time Interaction [$F(2,37) = 0.290$, $F(1,37) = 3.660$, $F(2,37) = 0.06$; respectively, all n.s.; see Fig 2B).

2-4-2. Experiment 2: Operant Sessions and Microdialysis

Cue Conditioning/Operant Sessions

A three-way ANOVA performed on the number of lever responses during MDMA and non-reinforced sessions showed significant effects of Group ($F(4,88)=7.82$; $p < 0.0001$), Treatment Level ($F(1,88)=119.5$; $p < 0.0001$), Days ($F(2,176)=34.63$; $p < 0.0001$), Group X Treatment Interaction ($F(4,88)=4.19$; $p < 0.01$), Group X Days Interaction ($F(8,176)=3.45$; $p < 0.01$), and Treatment Level X Days Interaction ($F(2,176)=13.91$; $p < 0.0001$). The Group X Treatment Level X Days Interaction effect was not significant ($F(8, 176)=1.91$; n.s.). Main findings of posthoc tests revealed significantly higher lever responses during non-reinforced compared to MDMA sessions, and a gradual decrease in responding over the 3 days of non-reinforced sessions while MDMA responding remained stable across MDMA sessions. In addition, non-reinforced responding in the MDMA Alone, MDMA + Noise and Noise Alone groups were significantly greater on the first non-reinforced session compared to all other groups. No significant group differences were detected during MDMA sessions (see Fig 3).

Microdialysis Test Sessions

A one-way ANOVA on baseline means (± SEM) of uncorrected DA and 5-HT nM levels showed no significant differences in absolute basal DA levels between experimental

groups [F(5,49)=1.021 and F(5,49)=1.434, respectively, both *n.s.*]. Therefore, data conversion to percent of baseline did not distort between-group comparisons.

Dopamine.

A two-way repeated measures ANOVA (Group x Time) showed significant effects of Group [F (5,44) = 4.632, $p = 0.002$], Time [F(5,220) = 6.869, $p < 0.001$] and Group X Time Interaction [F (25,220) = 2.601, $p < 0.001$]. Posthoc tests revealed that self-administered MDMA on test day resulted in a significant DA increase from baseline (last interval) in all groups ($p < 0.05$). In addition, the magnitude of DA response during the first 10-min interval after MDMA injection was greatest in the MDMA + Music group compared to all others ($p < 0.05$; see Fig. 4A). No significant differences in DA were observed in groups receiving saline at test (see Fig 4B).

Serotonin.

A two-way repeated measures ANOVA (Group x Time) showed significant Group [F (5, 44) = 5.758, $p < 0.001$], Time [5-HT: F (5,220) = 11.684, $p < 0.001$] and Interaction effects [F (25,220) = 3.994, $p < 0.001$]. Post hoc tests revealed that only the MDMA + Music group and MDMA Alone group showed an increase in 5-HT concentrations compared to baseline ($p < 0.05$). The MDMA + Music group had a significantly greater 5-HT response during the first 10-min interval post-MDMA injection compared to all other groups ($p < 0.05$) and higher than all groups except for the MDMA Alone group for the remainder of the test session ($p < 0.01$; see Fig. 5A). No significant differences in 5-HT were observed in groups receiving saline at test (see Fig 5B).

Locomotor Activity.

A two-way repeated measures ANOVA (Group x Time) showed significant Time [$F(5, 220)=4.602$, $p = 0.001$] and Interaction effects [$F(25,220)=1.964$, $p = 0.006$], but no significant Group differences [$F(5,44)=1.887$, *n.s.*]. Post hoc tests revealed that the MDMA Alone and Saline Alone groups showed significantly lower locomotor activity levels during some baseline intervals ($p < 0.05$). Additional comparisons showed that the MDMA + Music group was the only group showing a significant enhancement in locomotor activity from baseline (last baseline interval) to the first 10-min post-injection interval ($p < 0.001$). As can be seen in Fig 6A, locomotor activity during the same 10-min period was significantly higher in the MDMA + Music group than all other groups ($p < 0.05$) except for MDMA + Noise. No significant changes were observed in any group after a non-reinforced lever press, though the Saline Alone group showed lower baseline activity prior to saline infusion (see Fig 6B).

CONDITIONED PLACE PREFERENCE GROUPS

Groups	N-size	Compartment 1 (Days 1,3, & 5)	Compartment 2 (Days 2, 4, & 6)
1	17	MDMA + Music	Saline Alone
2	13	MDMA + Noise	Saline Alone
3	30	MDMA Alone	Saline Alone
Control			
4	14	Saline Alone	Saline Alone
5	10	Music Alone	Saline Alone
6	16	Noise Alone	Saline Alone

Table 1: Conditioned Place Preference Groups/Experiment 1

Rats received MDMA (1.5 mg/kg s.c.) 5 minutes before being placed in the chamber with music, white noise, or no additional sound. On alternate days an equal volume of saline (0.1 ml) was given. Control groups were pretreated with saline every day prior to conditioning and exposed to either music, white noise, or no additional sound every other day.

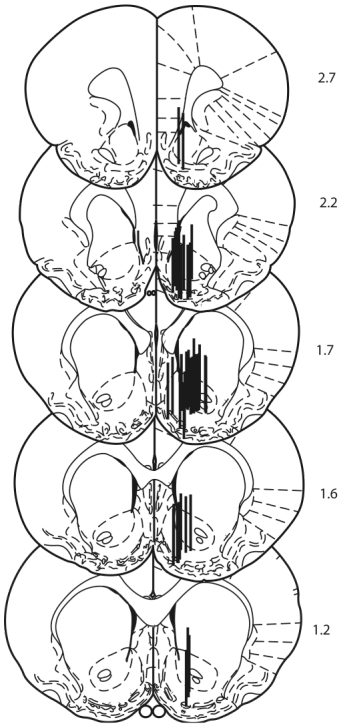


Figure 1: Histological Diagram

Drawings are representative of active membrane regions of dialysis probes in the core and shell subterritories of nucleus accumbens (NAcc). Illustrations drawn with assistance (Paxinos and Watson, 1997). Coronal sections of probe traces ranged from +2.70 mm to +1.20 mm anterior to bregma.

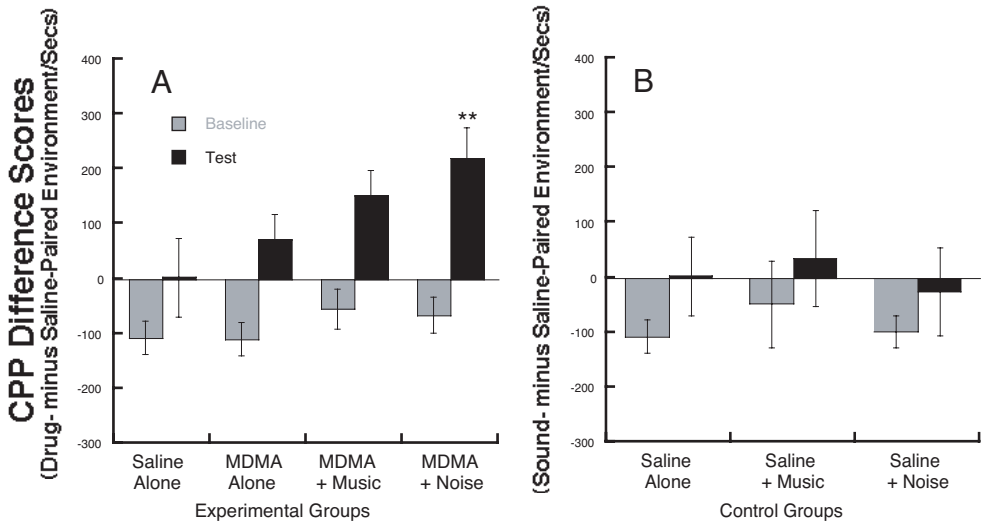


Figure 2: Conditioned Place Preference

Data are represented mean (+/- *SEM*) difference scores. For MDMA receiving groups, difference scores were time spent in the drug-paired compartment minus the saline-paired compartment before and after conditioning trials. For Control groups receiving saline on both sides, difference scores were time spent in the sound-paired compartment minus the time spent in the compartment with no additional sound. **(A)** Significant CPP was observed only in the MDMA + Noise group (** = $p < 0.01$ compared to Saline Alone). **(B)** No significant CPP was produced by Saline Alone, Music Alone or Noise Alone treatments.

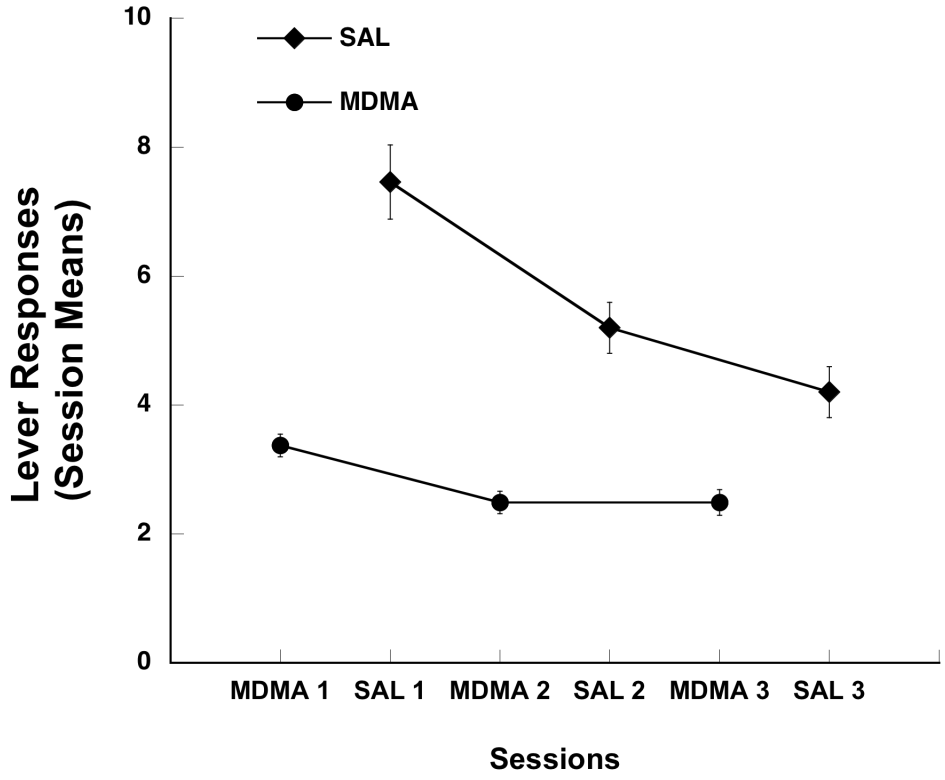


Figure 3: Lever responses during cue conditioning/operant sessions

Data show combined means for all groups. One three occasions, rats had access to MDMA in the presence of music (MDMA + Music, n=11), white noise (MDMA + Noise; n=10), or with no added auditory stimuli (MDMA Alone; n=29). On alternating days, lever responses were not reinforced, but animals were in the presence of music (Music Alone; n=6), white noise (Noise Alone; n=7), or no added sound (Saline Alone; n=37).

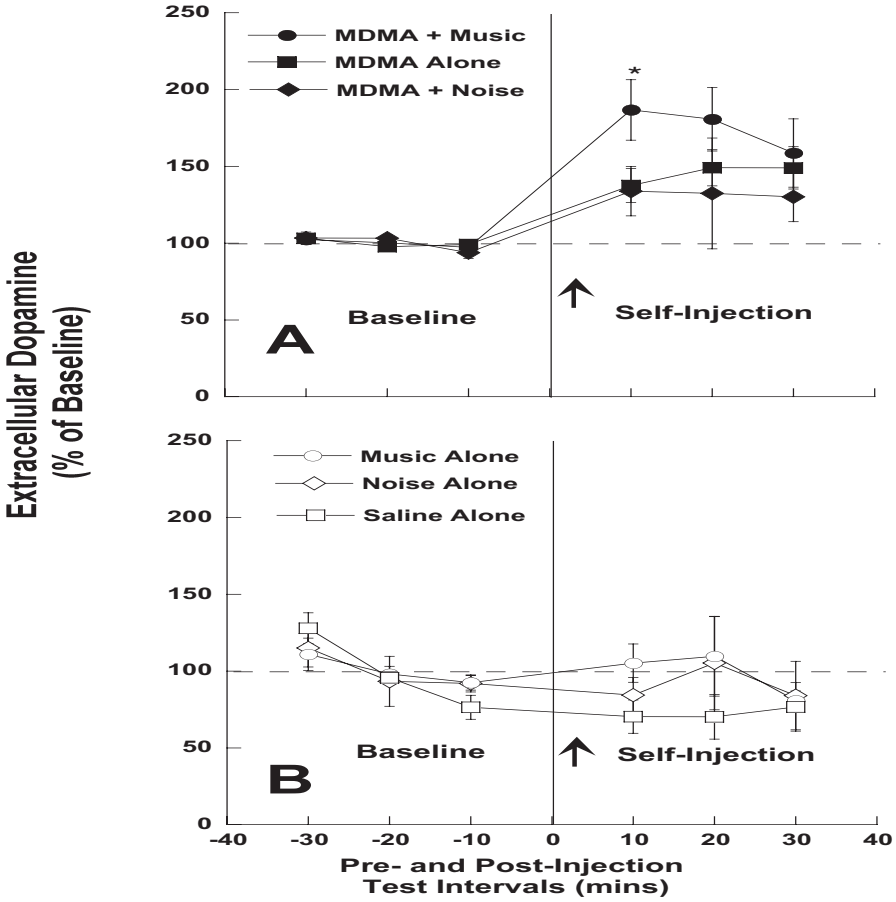


Figure 4: NAcc DA before and after voluntary intake of MDMA (A) and saline (B)

Timeline represents DA levels (% of baseline mean +/- *SEM*) at 10 min intervals, from 30 min before to 30 min after a single operant response (e.g., MDMA 1.5 mg/kg, or saline 0.1 ml). **(A)** Following MDMA self-administration (1.5 mg/kg), all three groups showed significantly greater enhancement in NAcc DA compared to baseline following MDMA self-administration. In addition, immediately after MDMA injection, NAcc DA was significantly higher in the presence of music (MDMA + Music; n=11) compared to white noise (MDMA + Noise; n=10) or no additional sound (MDMA Alone; n=9) (* = $p < 0.05$). **(B)** There were no significant changes from baseline following a non-reinforced operant response in the presence of music (Music Alone; n=6), white noise (Noise Alone; n=7) or no additional sound (Saline Alone; n=7). No other group differences were observed during the test session.

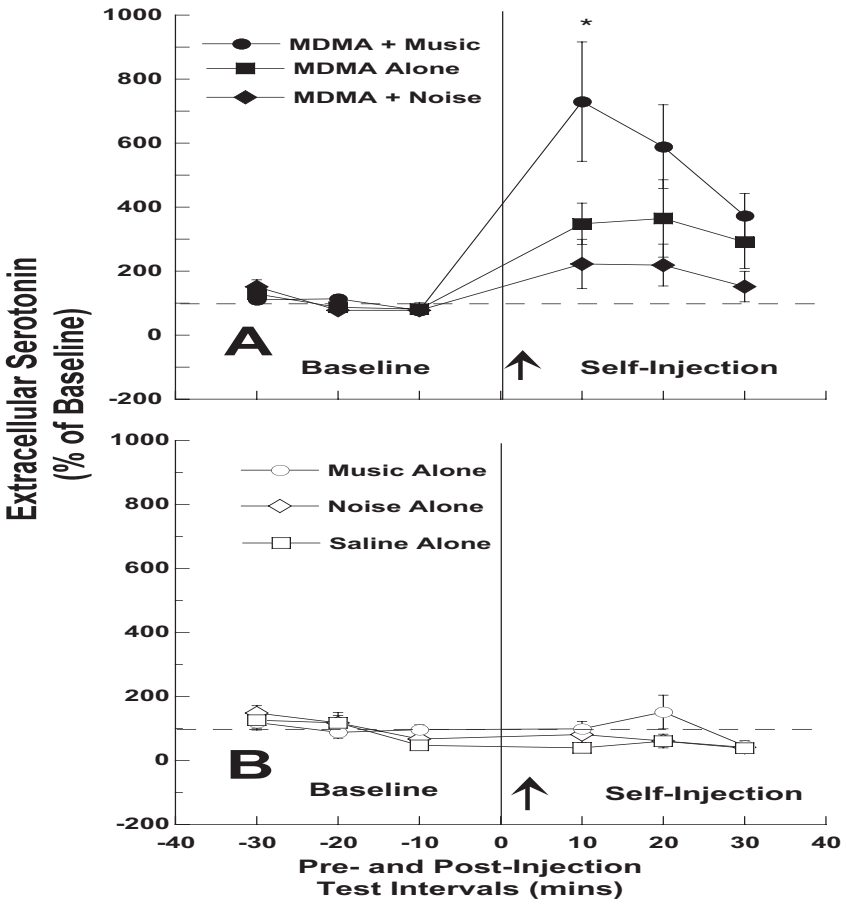


Figure 5: NAcc 5-HT before and after self-administered MDMA (A) and saline (B)

Timeline represents 5-HT levels (% of baseline mean +/- *SEM*) at 10 min intervals, from 30 min before to 30 min after a single operant response (e.g., MDMA 1.5 mg/kg or saline 0.1 ml). **(A)** Following MDMA self-administration (1.5 mg/kg), the groups receiving MDMA in the presence of music (MDMA + Music; n=11) and in the absence of additional sound (MDMA Alone; n=9), showed a significant increase in NAcc 5-HT compared to baseline following MDMA self-administration, while the MDMA + Noise group (n=10) did not. In addition, immediately after MDMA injection, NAcc 5-HT was significantly higher in the MDMA + Music group compared to MDMA + Noise or MDMA Alone (* $p < 0.05$). **(B)** There were no significant changes from baseline following a non-reinforced operant response in the Music Alone (n=6), Noise Alone (n=7) and Saline Alone (n=7) groups. No other group differences were observed during the test session.

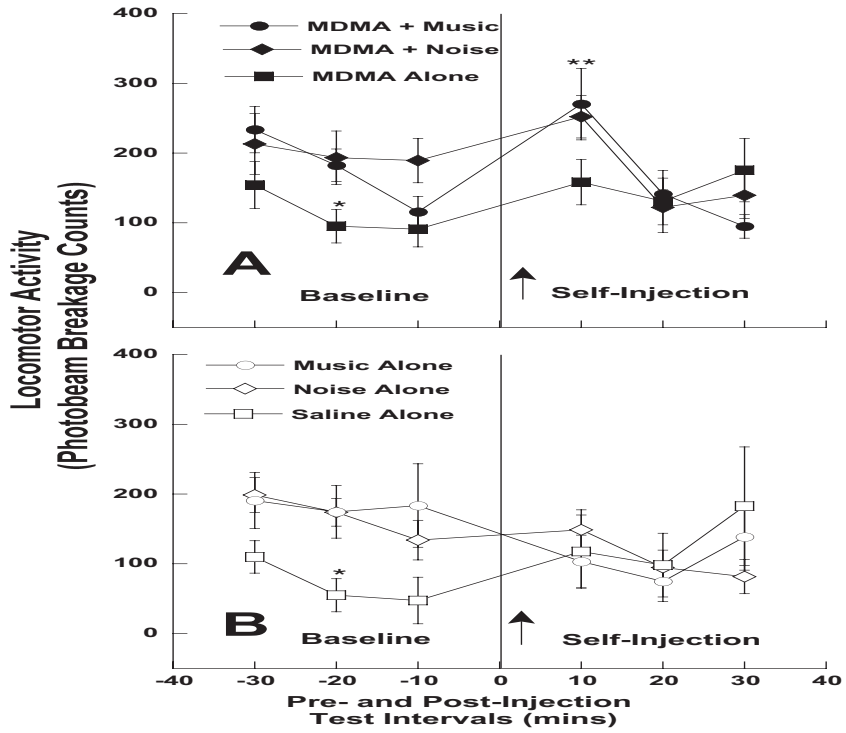


Figure 6: Locomotor activity before and after self-administration of MDMA (A) and Saline (B) on microdialysis test day

The graph represents photobeam breakage means (+/- *SEM*). **(A)** The MDMA Alone group had significantly less baseline locomotor activity compared to all other MDMA groups (* $p < 0.05$). At the first post-MDMA interval, the MDMA + Music group was the only group showing significantly increased locomotor activity compared to the baseline interval just prior to MDMA injection (** = $p < 0.001$). **(B)** The Saline Alone group showed significantly lower baseline activity than all other control groups (* = $p < 0.05$). No change in locomotor activity was detected for any group after the non-reinforced lever response.

2-5. DISCUSSION

Low doses of MDMA (1.5 mg/kg/session) in the presence of white noise resulted in a significant conditioned place preference (CPP). Since white noise paired with saline did not produce CPP, these results suggest rewarding effects of white noise only in combination with MDMA. However, the trend of increasing preference for MDMA + Music and MDMA Alone should be noted. It is possible that with additional training or by changing some aspect of the music, significant CPP would be detected for the MDMA + Music group. Previous studies have found that MDMA administration results in a dose dependent CPP in rats, with optimal doses ranging from 2.0 –10.0 mg/kg (Bilsky et al., 1990; Marona-Lewicka et al., 1996; Meyer et al., 2002). However, other non-drug factors, such as housing conditions, have been shown to influence the ability of MDMA to produce a significant CPP, with isolated animals showing a more pronounced CPP than group-housed rats (Herzig et al., 2005; Meyer et al., 2002). The impact of the home cage environment on the reinforcing properties of MDMA within the CPP context suggests that non-drug factors present prior to drug use can play a critical role in the subjective rewarding value of MDMA drug experiences. Our findings indicate that non-drug factors, such as auditory stimulation, during MDMA use can also affect its conditioned rewarding value.

In Experiment 2, animals had access to MDMA under differing stimulus conditions during a total of three sessions that alternated daily with non-reinforced (saline) sessions. However, since acquisition of MDMA self-administration requires a lengthy training period of daily consecutive sessions (Reveron et al., 2006; Schenk et al., 2003), the self-administration opportunity of the present study was too limited to enable full acquisition

of MDMA self-administration behavior. Indeed, the aim of the current study was not to establish behavioral demonstration of MDMA reward through self-administration behavior, but to allow voluntary intake of MDMA in a distinctive environment. Nevertheless, consistent with previous reports showing the eventual demonstration of MDMA reinforcement (Reveron et al., 2006), rats maintained a stable number of MDMA-reinforced lever responses during these sessions. Though the number of lever responses for saline on alternate days was significantly higher than for MDMA, this observation is likely to be due to the experimental procedures of alternating reinforcement conditions. For example, one possible explanation is that early "extinction" responding on non-reinforced days accounted for the higher rate of non-reinforced operant responses. Indeed, this phenomenon has also been shown with a similar schedule of cocaine and saline self-administration/conditioning trials (Ikegami et al., 2007).

In the subsequent *in vivo* microdialysis test session, animals were exposed to the same stimulus conditions as during the operant sessions and allowed to lever press for MDMA or saline. NAcc DA was significantly increased from baseline levels in all groups receiving MDMA (1.5 mg/kg, i.v.), but most dramatically when MDMA was self-administered in the presence of music. MDMA also increased 5-HT levels, but significant elevations were only observed in the MDMA alone and MDMA + Music group. The MDMA + Noise group showed a slight, but non-significant rise in 5-HT ten minutes after the self-infusion. This finding suggests that the presence of music in combination with MDMA mimics a higher dose of MDMA. If so, this pattern of DA and 5-HT responses to MDMA is consistent with a previous study in which a higher dose of MDMA (5.0 mg/kg, i.p.), significantly increased both NAcc DA and 5-HT levels, but at a

lower dosage (2.5 mg/kg, i.p.), NAcc DA was significantly increased, but 5-HT levels were not (O'Shea et al., 2005).

As can be seen in Figs 4B and 5B, no significant changes in DA or 5-HT were observed in response to music or white noise alone, indicating that the enhancement seen in the MDMA + Music group was synergistic. While the mechanisms underlying this effect remain unknown, evidence indicates a significant influence of auditory stimulation in the presence of MDMA and on monoamines levels. For example, significant changes in electrocortical activity were observed in rats receiving MDMA with additional acoustic stimulation compared to animals that received the same dose of MDMA without additional noise. In addition, no change in electrocortical activity was observed in animals exposed to loud white noise (95 dB) as compared to control animals that received no additional sound (Iannone et al., 2006). Other work has shown a relationship between noise alone and monoamine metabolite (DOPAC, HVA, and 5-HIAA) levels in the striatum of mice (Tsai et al., 2005), though the intensity of white noise (110 dB) was much higher than that of the present study (70 dB).

It is conceivable that changes in DA and 5-HT shown in the present study could be due to increased stress in response to auditory stimulation. Indeed, noise above 80 dB can raise the pain threshold in mice, suggesting that noise at this intensity may induce a stress response (Chen, 2002). However, decibel levels in the present study did not exceed 75 dB and animals exposed to music or white noise in the absence of MDMA did not show enhanced NAcc DA or 5-HT.

Though it seems likely that the changes observed in the present study were not due to auditory stimulation-induced stress, it should be noted that noise could increase MDMA-induced toxicity. For instance, nigrostriatal DA damage was enhanced in mice following exposure to loud noise combined with MDMA (Gesi et al., 2004). In addition, mice given methamphetamine (75 mg/kg) while exposed to loud music (95 Db) experienced more seizures than mice given the same dose of methamphetamine in silence (ambient noise; 55 Db) or in the presence white noise (95 Db) (Morton et al., 2001). These findings call for further research to determine neural mechanisms underlying synergistic effects of MDMA and auditory stimuli.

During the dialysis test session, MDMA + Music was the only group that showed a significant enhancement in locomotor activity from the last baseline interval to the first post-injection interval, correlating with the high release of DA and 5-HT seen in this group. Previous studies have shown that MDMA enhances locomotor activity in a dose-dependent manner and is capable of producing locomotor sensitization after repeated administration (Callaway et al., 1992; Spanos and Yamamoto, 1989). The dopaminergic and serotonergic systems have both been shown to mediate MDMA-induced locomotor hyperactivity (Bankson and Cunningham, 2001; Callaway et al., 1990; Gold et al., 1989). Furthermore, like other psychostimulants, MDMA can produce conditioned locomotion after the drug has been repeatedly administered and paired with environmental cues. Ultimately, conditioned stimuli (environmental cues) alone cause an increase in locomotor activity even when the drug is not present and can act as a predictor for the animal of the drug availability (Gold et al., 1989). Therefore, in the MDMA + Music group, it is conceivable that music acted as a conditioned stimulus that further enhanced MDMA-stimulated locomotion.

The large increases in DA and 5-HT levels observed in the MDMA + Music group may have involved inherent properties of the music. Music has variations in rhythm, pitch, loudness, and frequency that may cause an increase in arousal levels in animals. For example, avian studies have shown that rhythmic auditory stimuli, but not human speech, increased physiological arousal through enhanced noradrenaline levels (Toukhsati et al., 2005). The reticular activating system is known to mediate arousal levels and responds to changes in the organism's acoustic environment (Siegel, 1979). Therefore, it is conceivable that the reticular formation may become more sensitive to changes in auditory input in an MDMA-treated animal. On the other hand, since white noise contains an equal amount of energy per frequency band, it may suppress arousal and/or have the opposite effect of music. Our finding that the only group not showing significant MDMA-induced enhancement of 5-HT was the MDMA + Noise condition provides some support to that notion.

Evidence also suggests that music influences the same pathways in the brain that are activated by natural rewards, such as food and sex, as well as drugs of abuse. Music has been shown to improve dopaminergic neurotransmission and increase neostriatal DA concentrations in spontaneously hypertensive rats (SHR) (Sutoo and Akiyama, 2004). A human fMRI study showed the mesolimbic dopaminergic system, including the NAcc and VTA, are highly activated and tightly connected during music processing (Menon and Levitin, 2005). The hypothalamus, an important area for the physiological and autonomic response to emotional stimuli was also activated when subjects listened to pleasant music (Blood and Zatorre, 2001). Taken together, these findings indicate that music and MDMA may work on common neural networks involving reward and arousal

and most likely influence each other. Since Ecstasy is often taken in environments rich in sensory stimulation, understanding the impact of music and other auditory stimuli during the MDMA experience is a crucial for evaluating MDMA effects and the potential for neurotoxicity. [1]

[1] Previously published data in chapter 2 of this dissertation from Feduccia, A.A., Duvauchelle, C.L., 2008. Auditory stimuli enhances MDMA-conditioned reward and MDMA-induced nucleus accumbens dopamine, serotonin and locomotor responses. Brain Res Bull 77, 189-196.

CHAPTER 3: HEAT INCREASES MDMA-ENHANCED NUCLEUS ACCUMBENS SEROTONIN AND BODY TEMPERATURE, BUT NOT MDMA SELF-ADMINISTRATION

3-1. ABSTRACT

There is concern that hot environments enhance adverse effects of 3,4-methylenedioxymethamphetamine (MDMA or "Ecstasy"). In this study, long-term (4-wks) daily MDMA self-administration sessions and an MDMA challenge test were conducted with rats under normal and high thermal conditions (23° or 32°C). During MDMA self-administration sessions, activity and body temperature were increased by heat and/or MDMA experience, while MDMA self-administration rates were comparable between thermal conditions. At the MDMA challenge test (3.0 mg/kg, i.v.), *in vivo* microdialysis showed nucleus accumbens serotonin (NAcc 5-HT) and dopamine (DA) responses were significantly increased, but in the heated environment 5-HT responses were significantly greater than at room temperature. DA responses did not show significant thermal effects. Though the heated environment did not acutely boost MDMA intake, exaggerated NAcc 5-HT responses to MDMA may result in 5-HT depletion; a condition associated with Ecstasy use escalation and neural dysfunctions altering mood and cognition.

3-2. INTRODUCTION

The amphetamine derivative, 3,4-methylenedioxymethamphetamine (MDMA), is a major component of Ecstasy, a commonly abused drug that is particularly popular among electronic dance music enthusiasts and club goers. Nightclubs and raves feature loud

techno music, laser lights, crowded and hot social environments that attract Ecstasy users. Indeed, human subjects report a higher euphoric state when taking the drug in sensory rich environments, as compared to people who take the drug in less stimulating contexts (Bedi and Redman, 2006; McElrath and McEvoy, 2002; Parrott, 2004). Though MDMA-induced lethality is rare, the use of MDMA is associated with several negative consequences such as cardiac arrhythmias, renal failure, rhabdomyolysis, cognitive deficits, negative affect and aggressive bias (Curran et al., 2004; Hall and Henry, 2006; Indlekofer et al., 2009; Kalant, 2001). It has been proposed that elevated ambient temperatures, such as those encountered in rave venues, can exacerbate MDMA-induced temperature-increasing effects and the likelihood of adverse drug effects (Parrott, 2004; Parrott et al., 2002).

MDMA increases extracellular levels of both serotonin (5-HT) and dopamine (DA) (Gudelsky and Nash, 1996; Kankaanpaa et al., 1998). Several studies show the increase in magnitude is greater for 5-HT (Baumann et al., 2008; Verrico et al., 2007), though others report greater DA effects (Gough et al., 1991). As the specific receptor subtypes, D_1 and 5-$HT2_A$ have been shown to influence thermoregulatory responses (Benamar et al., 2008; Shioda et al., 2008), the combined enhancement of 5-HT and DA may contribute to MDMA's unique effects on thermoregulation.

In animal studies, MDMA has been reported to induce both hypothermia and hyperthermia, depending on several factors including MDMA dosage, amount of MDMA experience, and environmental ambient temperature (Malberg and Seiden, 1998). High MDMA doses (20 mg/kg, i.p.) reliably produce hyperthermia in rats (Benamar et al., 2008), but a heated environment (e.g., 30°C) can elicit hyperthermia from a lower

MDMA dose (10 mg/kg, i.p.) (Hargreaves et al., 2007). In rodents, the magnitude of the hyperthermic response has been tightly correlated with MDMA-induced 5-HT depletion in various brain regions (Broening et al., 1995; Malberg and Seiden, 1998; Sanchez et al., 2004).

MDMA is thought to be of lower reinforcement value than other abused drugs, such as cocaine (Lile et al., 2005), but is reliably self-administered by rodents (e.g., Daniela et al. 2004). Studies have reported that self-administered MDMA results in experience-dependent changes in MDMA-motivated reinforcement, activity levels and temperature regulation (Daniela et al., 2004; Ratzenboeck et al., 2001; Reveron et al., 2006; Schenk et al., 2003; Schenk et al., 2007).

In the present study, operant chambers were maintained at either 23° C (e.g., Room Temperature) or 32° C (e.g., High Temperature). During 20 daily 2-hr sessions, operant-trained rats had the opportunity to press a lever that delivered either MDMA or saline. Following completion of the self-administration sessions, a 1-hr MDMA Challenge test was conducted using *in vivo* microdialysis techniques. For this test, animals were placed in the operant chamber under the same thermal conditions as during self-administration sessions and were allowed to elicit a single lever response that resulted in either MDMA- (3.0 mg/kg) or saline (0.1 ml). Dialysate samples collected in 10-min intervals enabled the determination of NAcc 5-HT and DA levels under differing thermal conditions.

3-3. MATERIALS AND METHODS

3-3-1. Experimental Procedures

Animals

Male Sprague-Dawley rats (5 weeks old, Charles River Laboratories, Inc., Wilmington, MA) were housed in an animal colony (ambient temperature 22 +/-1 C) in clear cages with a 12:12 reverse light dark cycle. Laboratory food pellets and water were available ad libitum. Rats underwent 2 weeks of handling before the start of the experiment. All protocols and procedures were in accordance with the Guide for the Care and Use of Laboratory Animals (U.S. Public Health Service, National Institute of Health) and the Institutional Animal Care and Use Committee (IACUC) at the University of Texas at Austin.

3-3-2. Apparatus

Operant Chambers

All experimental sessions were conducted in operant chambers (28 x 22 x 21 cm) located within sound-attenuating compartments (Med Associates, St. Albans, VT). A house light within the chamber and a single retractable lever located on the right wall were activated at the start of each session. During self-administration sessions, the catheter inlet from each rat was connected to spring-covered tubing (Plastics One, Roanoke, VA) attached to a drug swivel mounted on a balancing arm. Tygon tubing attached at the other end of the drug swivel extended to a 10 cc syringe, containing MDMA or saline solution, that was mounted on a motor-driven syringe pump (Razel, St. Albans, VT). Each lever press activated the syringe pump that delivered 0.1 ml of saline or MDMA solution. A stimulus light directly above the lever remained on for the duration of the injection (6 sec). Three

sets of photocells, spaced evenly apart across the lower front and back walls of the chamber detected photobeam breakages that were used as an index of locomotor activity. Lever presses and photobeam breakage were recorded during each session by a Med Pentium 100 MHZ computer equipped with Med-PC software.

3-3-3. Thermal Control System

Ceramic infrared heat emitters (Big Apple Herpetological Inc., Holbrook, NY) controlled by a proportional thermostat (Big Apple Herpetological Inc., Holbrook, NY) and digital thermometers (Fisher Scientific, Pittsburgh, PA) were used to regulate and maintain operant chamber temperatures at either 23° or 32° C (+/- 1° C) during experimental sessions.

3-3-4. Food Training

Rats were trained to lever press using food reward (45 mg sucrose pellets; P.J. Noyes, Lancaster, NH) and a fixed ratio (FR1) schedule of reinforcement. Animals were food restricted (approximately 6 g of laboratory rat chow per day, adjusted to maintain weight) until lever responding was acquired. Food-reinforced operant sessions were 10 min/day for approx 8 days.

3-3-5. Surgical Procedures

For intravenous drug or saline delivery and *in vivo* microdialysis procedures, jugular catheterization and stereotaxic surgery for guide cannula implantation was performed as previously described (Feduccia and Duvauchelle, 2008). During the surgical procedure, rats were anesthetized with 2.5% isoflurane (VetEquip, Pleasanton, CA) vaporized in

oxygen at a flow rate of 0.8 L/min. Coordinates for the unilateral guide cannula (21 g; Plastic One, Roanoke, VA) aimed above the NAcc were as follows: AP: + 0.2 mm, ML: +/- 0.12 mm, DV: -2.5 mm. After surgery, Rimadyl (5 mg/kg, s.c.) was administered for prophylactic pain relief. To maintain patency, jugular catheters were flushed daily with 0.1 ml of 0.9% saline containing 1 U/ml heparin and 67 mg/ml Timentin. After one week, the Timentin component was removed from the solution, though daily catheter flushing continued throughout the duration of the experiment.

3-3-6. MDMA

(+/-) 3,4-methylenedioxymethamphetamine HCl (MDMA) (NIDA Drug Inventory Supply and Control Program; RTI International, Research Triangle Park, NC) was used in this experiment. MDMA was dissolved in isotonic saline solution (0.9 %) in the appropriate dose concentrations according to the weights of the animals.

3-3-7. Groups and Experimental Procedures

One week after surgery, animals were randomly assigned to one of four groups: (1) MDMA Room Temperature, (2) MDMA High Temperature, (3) Control Room Temperature, or (4) Control High Temperature. Room Temperature groups had daily access to MDMA or Saline in an operant chamber maintained at 23° C (+/- 1°), while sessions for the High Temperature groups were conducted in operant chambers maintained at 32° C (+/- 1°). The core temperature of each animal was assessed before and after every self-administration session using a 7001H model microcomputer thermometer (Physitemp, Clifton, NJ).

3-3-8. Self-Administration Procedures

To achieve optimal MDMA self-administration behavior, as previously reported (Reveron et al., 2006; Schenk et al., 2007), the unit dose of MDMA was set at 1.0 mg/kg/inj for the first 10 sessions ("Acquisition"), followed by 0.5 mg/kg/inj for the last 10 days ("Maintenance"). Control groups had access to saline injections of the same volume of infusion (0.1 ml/inj) for the entire 20 self-administration sessions (5 days/week, weekends off). When animals were placed in the operant chamber and at the start of the session, the chamber remained dark and the lever was unavailable for a 30-min habituation period. After this interval, the house light illuminated and the lever was inserted into the chamber. Animals then had access to the lever and the opportunity to administer MDMA or saline injections for 2 hr/session.

3-3-9. Microdialysis Procedures

In vitro recovery calibration

Microdialysis probes were constructed as previously described (Duvauchelle et al., 2000) with an active membrane length of 2.5 mm at the probe tip. For each probe, recovery values were calculated by comparing the peak heights of samples to those from a standard as previously described (Ikegami et al., 2007). The mean (± SEM) recovery of probes used in the experiment was 11.55 ± 0.39% for DA and 10.79 ± 0.44% for 5-HT.

Probe implantation

At least 12 hours prior to the MDMA Challenge and *in vivo* microdialysis test, animals were briefly anesthetized with isoflurane and implanted with a microdialysis probe through their indwelling guide cannula. Probes extended 6.25 mm past the end of the guide for placement within the NAcc. After placement, artificial cerebral spinal fluid

(ACSF) was pumped through the probe at a speed of 0.2 μl/min with a 1.0 ml gastight Hamilton 1000 series syringe mounted on a syringe pump (Razel®, Model A). The pump speed was increased to 1.60 μl/min one hour before the test session.

3-3-10. MDMA Challenge and Microdialysis Test Session

24 hrs after the last self-administration session (e.g., Day 21) animals were placed within the operant chamber and experienced the 30-min habituation period, thermal and drug group conditions identical to those assigned during self-administration sessions. The only differences in this test session were that (1) animals were allowed only a single operant response resulting in MDMA (3.0 mg/kg) or saline (0.1 ml) and lever was retracted for the remainder of the session, and (2) brain dialysate samples were collected throughout the session at 10-minute intervals (3 10-min baseline and 6 10-min post-injection).

3-3-11. Analysis of DA and 5-HT

To determine *in vivo* extracellular DA and 5-HT concentrations, dialysate samples were analyzed by high performance liquid chromatography and electrochemical detection (HPLC-EC; Shizeido Capcell C-18 narrow bore column, ESA model 5200A Coulochem III Detector, Model 5041 cell (oxidizing potential set to +200 mV, sensitivity 100 pA) and a Model 5020 Guard Cell (potential 400 mV); ESA, Inc., Chelmsford, MA). The mobile phase contained sodium dihydrogen phosphate (75 mM), citric acid (4.76 mM), SDS 1 g/l, EDTA (0.5 mM), MeOH 8% and acetonitrile 11% (v/v), pH 5.6. An ESA model 420 dual pistons HPLC pump circulated mobile phase through the system at a rate of 0.2 ml/min. An ESA Model 500 data station controlled the programs and data collection. The detection limit of DA was 0.1 nM and 0.12 nM for 5-HT, both with a

signal/noise ratio of 3:1. Uncorrected basal concentrations of NAcc DA ranged from 0.443 to 0.479 nM and basal NAcc 5-HT ranged from 0.126 to 0.189 nM.

3-3-12. Histology

At the conclusion of the experiment, animals were sacrificed and brains were collected, stored in 10% formalin/30% sucrose solution, sectioned (48 μm) and stained with cresyl violet for histological analysis to confirm placement within the NAcc (see Fig 1).

3-3-13. Statistical Analyses

One-way ANOVAs were used to compare group differences in core temperature before and after self-administration across Acquisition and Maintenance sessions and before and after the MDMA Challenge test. Two-way repeated measures ANOVA (Group x Session Day) was performed on number of daily lever responses and daily locomotor activity (e.g., photobeam breakages) during Acquisition and Maintenance intervals. T-tests (Independent Samples) were used to compare MDMA intake (total mg/kg) between MDMA Room and High Temperature groups and between Acquisition and Maintenance intervals (Paired Samples). DA and 5-HT nM concentrations collected during the MDMA Challenge test were converted to percent of baseline values and analyzed using 2-way repeated measures (Group X Time). Pearson's Correlation analyses were performed to determine relationships between the number of lever responses and core temperature changes during self-administration sessions. Post hoc analyses (Fisher's LSD) were used when justified by significant main effects.

3-4. RESULTS

3-4-1. Operant Sessions: Acquisition and Maintenance Phases

Lever Responses

Acquisition (Session Days 1-10). A two-way repeated measures ANOVA (Group x Session Day) on daily lever responses during Acquisition showed significant effects of Group [$F(3,28)=4.322$; $p<0.05$], Session Days [$F(9,252)=12.554$; $p<0.001$] and Group X Session Day Interaction [$F(27, 252)=4.775$; $p<0.001$]. Post hoc tests revealed that Control groups had significantly greater lever responses than MDMA groups on several occasions (see Fig 8 Panel A).

Maintenance (Session Days 11-20). Significant Group [$F(3,28)=5.360$; $p<0.01$] and Group x Session Day Interaction [$F(27, 252)=1.830$; $p<0.05$], but not Session Day [$F(9,252)=1.192$; *n.s.*] effects were detected. Post hoc tests revealed significantly greater lever responses in MDMA Groups compared to Controls during several sessions (see Fig 8 Panel B).

3-4-2. MDMA Intake (Acquisition vs. Maintenance)

No significant differences were detected in total MDMA intake (mg/kg) between the two MDMA thermal conditions during Acquisition [$t(14)=-0.879$; *n.s.*] or Maintenance [$t(14)=-0.542$; *n.s.*], therefore MDMA intake data was combined for comparison purposes. Paired samples t-test revealed that the total MDMA intake during Maintenance was significantly greater than during the Acquisition interval [$t(15)=-2.219$; $p<0.05$] (see insert Fig 8).

3-4-3. Core Temperature

Acquisition (Session Days 1-10). A one-way ANOVA performed on mean core temperature difference scores (core temperature after session minus core temperature before session) of all experimental groups showed no significant differences [$F(3,31)=0.993$; *n.s.*] (see Fig 9A)

Maintenance (Session Days 11-20). A one-way ANOVA performed on mean core difference scores detected significant differences [$F(3,31)=5.316$; $p< 0.01$]. Post hoc tests revealed the MDMA High Temperature group had significantly greater core temperatures self-administration sessions than MDMA and Control Room Temperature Groups ($p<0.01$), but not greater than the Control High Temperature group (see Fig 9B).

3-4-4. Correlation between Total Lever Responses and Core Temperature

Acquisition (Session Days 1-10). Correlation analyses (Pearson's Correlation) performed between lever responses and core temperature differences during Acquisition sessions revealed significant correlations for MDMA High Temperature group [$r=0.780$; $p=0.022$] but not MDMA Room Temperature [$r=0.67$; *n.s*] or Control conditions [Control Room Temperature: $r=-0.196$; Control High Temperature: $r=-0.492$; both *n.s.*] (see Fig 10A).

Maintenance (Session Days 11-20). A significant correlation between lever responses and change in core temperature was determined for both MDMA groups [MDMA High Temperature: $r= 0.713$; $p=0.047$; MDMA Room Temperature: $r= 0.864$; $p=0.006$] but not Control conditions [Control Room Temperature: $r=-0.083$; Control High Temperature: $r=-0.53$; both *n.s.*] (see Fig 10B).

3-4-5. Locomotor Activity

Acquisition (Session Days 1-10). A two-way ANOVA (Group X Session Days) performed on locomotor activity counts during lever access across Acquisition sessions showed significant Group [$F(3, 28)=8.455$; $p<0.001$] and Session Day effects [$F(9,252)=5.576$; $p< 0.001$], but not Group x Session Day Interaction [$F(27,252)=1.281$; *n.s.*]. Post hoc tests showed locomotor activity between MDMA groups was not significantly different and that MDMA-stimulated activity was significantly greater than their respective Controls during a few matched sessions. However, activity in the Control High Temperature group was significantly higher than Control Room Temperature levels during a few initial matched sessions (see Fig 11 Panel A).

Maintenance (Session Days 11-20). A two-way ANOVA detected significant Group [$F(3, 28)=12.372$; $p<0.001$], but not Session Day [$F(9,252)=0.820$; *n.s.*] or Group x Session Day Interaction effects [$F(27,252)=1.304$, *n.s.*]. Post hoc tests revealed that activity in the MDMA groups were significantly higher than their respective controls on several occasions, but no effects of ambient temperature were observed in either the MDMA or Control groups (see Fig 11 Panel B).

3-4-6. Microdialysis Test Session

Dialysate levels of DA and 5-HT in nM concentrations (uncorrected values) were converted to percent of baseline to enable MDMA response magnitude comparisons. Basal concentrations (uncorrected values) of NAcc DA ranged from 0.443 to 0.479 nM and basal NAcc 5-HT ranged from 0.126 to 0.189 nM. One-way ANOVAs performed on DA and 5-HT nM baseline means showed no significant differences between experimental groups [$F(3,31)=2.248$ and $F(3,31)=1.283$, respectively; both *n.s.*].

3-4-7. NAcc 5-HT

A two-way repeated measure ANOVA (Group x Time) showed significant Group [$F(3,28)=23.449$; $p<0.001$], Time [$F(8,224)=39.393$; $p<0.001$] and Group x Time Interaction effects [$F(24,224)=18.247$; $p<0.001$]. Post hoc analysis revealed that both MDMA groups showed significant enhancement of NAcc 5-HT from baseline ($p<0.001$), and that the magnitude of enhanced NAcc 5-HT in the MDMA High Temperature was significantly greater than all other groups at all post-injection time points ($p<0.01$). Control groups showed no significant variations across the testing period (see Fig 12A).

3-4-8. NAcc DA

A two-way repeated measures ANOVA (Group x Time) showed significant Group [$F(3,28)=7.692$; $p<0.001$], Time [$F(8,224)=12.009$; $p<0.001$], and Group x Time Interaction effects [$F(24,224)=5.213$; $p< 0.001$]. Post hoc tests revealed that MDMA (3.0 mg/kg, i.v.) on test day resulted in a significant NAcc DA increase from baseline in both MDMA groups ($p<0.001$). Post-injection increases in NAcc DA did not significantly differ between the MDMA Room Temperature and MDMA High Temperature groups. NAcc DA levels in the Control conditions were comparable across the testing interval (see Fig 12B).

3-4-9. Core Temperature

A one-way ANOVA performed on mean core temperature difference scores detected significant effects [$F(3,31)=4.548$, $p<0.01$]. Posthoc testing revealed that core

temperature in the MDMA High Temperature group increased to a significantly greater extent than all other groups (see Fig 13).

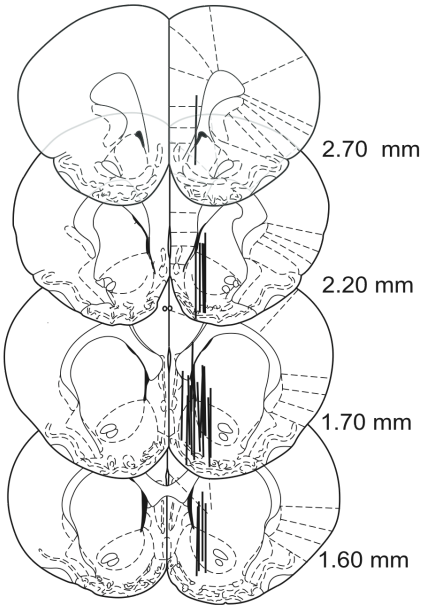


Figure 7: Histology

Illustrations depict active membrane regions of dialysis probes in the core and shell of the nucleus accumbens. Illustrations drawn with the assistance (Paxinos and Watson 1997). Coronal sections ranged from +2.7 to +1.6 mm anterior to bregma.

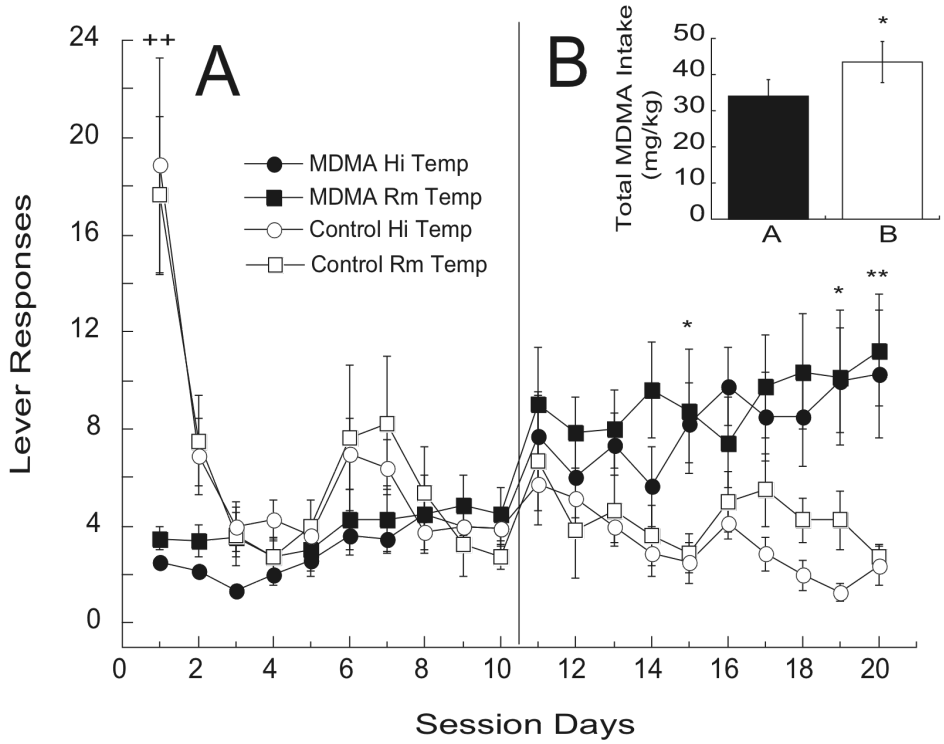


Figure 8: MDMA Self-Administration Sessions: Lever Responses and MDMA Intake

Mean daily lever responses and total MDMA intake (mg/kg) (+/- *SEM*) during (A) Acquisition (Session Days 1-10) and (B) Maintenance (Session Days 11-20). During Acquisition, MDMA dose was 1.0 mg/kg/inj and 0.5 mg/kg/inj during Maintenance sessions. Groups included: MDMA Room Temperature (n = 8), MDMA High Temperature (n = 8), Control Room Temperature (n =8), and Control High Temperature (n =8). Ambient temperature did not influence lever responding in either the MDMA or Control groups across all sessions. **(A)** All groups were trained to lever press for food reinforcement prior to MDMA sessions. The high number of responses for the first 2 sessions in both Control groups is a characteristic food extinction response pattern. Lever responses of the Control groups were significantly greater than MDMA groups during Acquisition (++ = both Control groups significantly greater than both MDMA groups @ $p < 0.01$). **(B)** Lever responses in the MDMA groups were significantly greater than Controls (*, ** = both MDMA groups significantly greater than both Control groups @ $p < 0.05$ and 0.01, respectively). Bar Insert: Total MDMA Intake (both MDMA groups combined) was significantly greater during Maintenance (B) compared to the Acquisition (A) interval.

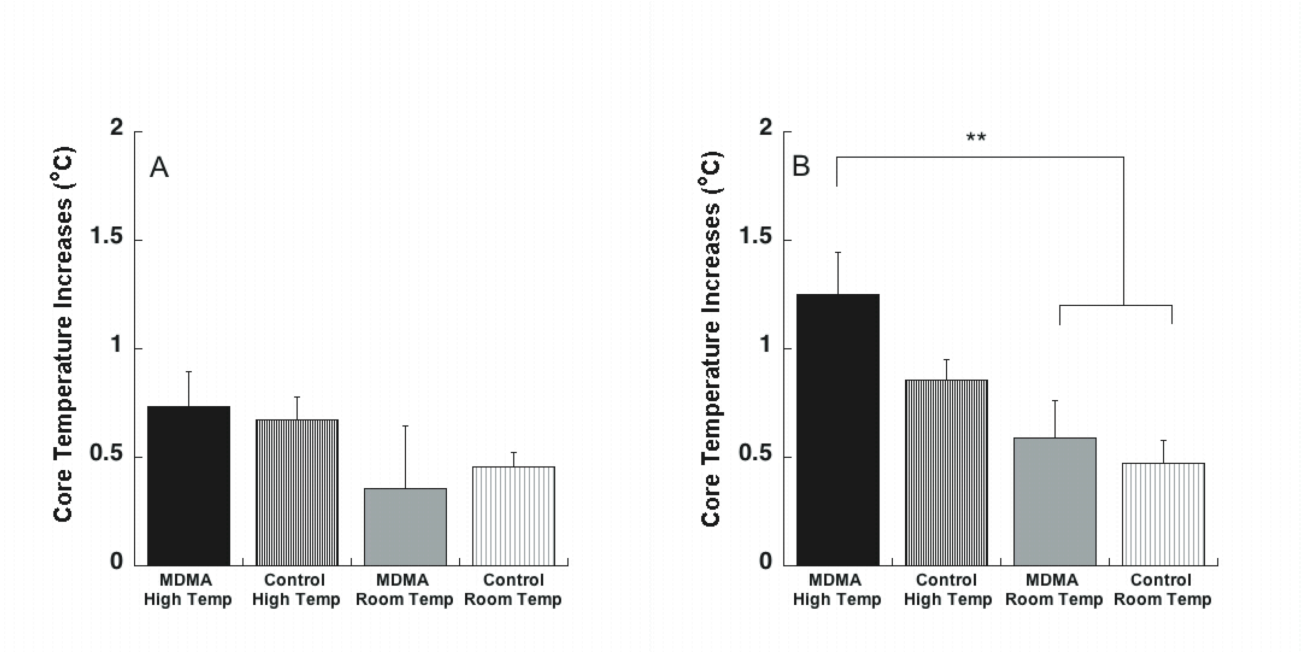
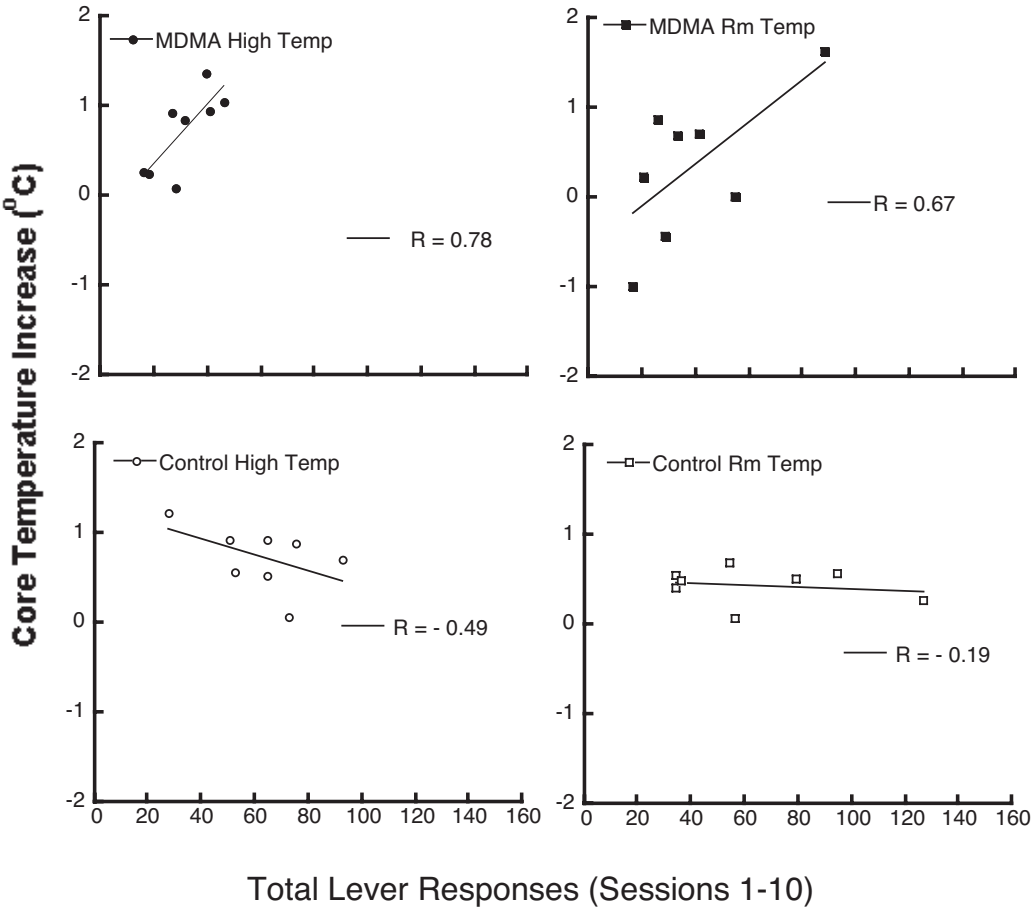


Figure 9: MDMA Self-Administration Sessions: Core Temperature Difference Scores

Data represent the mean (+/- *SEM*) of core temperature difference scores (After Session minus Before Session) during Acquisition and Maintenance sessions. **(A)** No significant core temperature differences between groups were observed during Acquisition. **(B)** Core temperature difference scores were significantly greater after MDMA self-administration in the MDMA High Temperature group than in the MDMA and Control Room Temperature groups, but not compared to the Control High Temperature group (** = significant difference @ $p < 0.01$).


MDMA High Temp
R = 0.78
MDMA Rm Temp
R = 0.67
Control High Temp
R = - 0.49
Control Rm Temp
R = - 0.19
Core Temperature Increase (°C)
Total Lever Responses (Sessions 1-10)

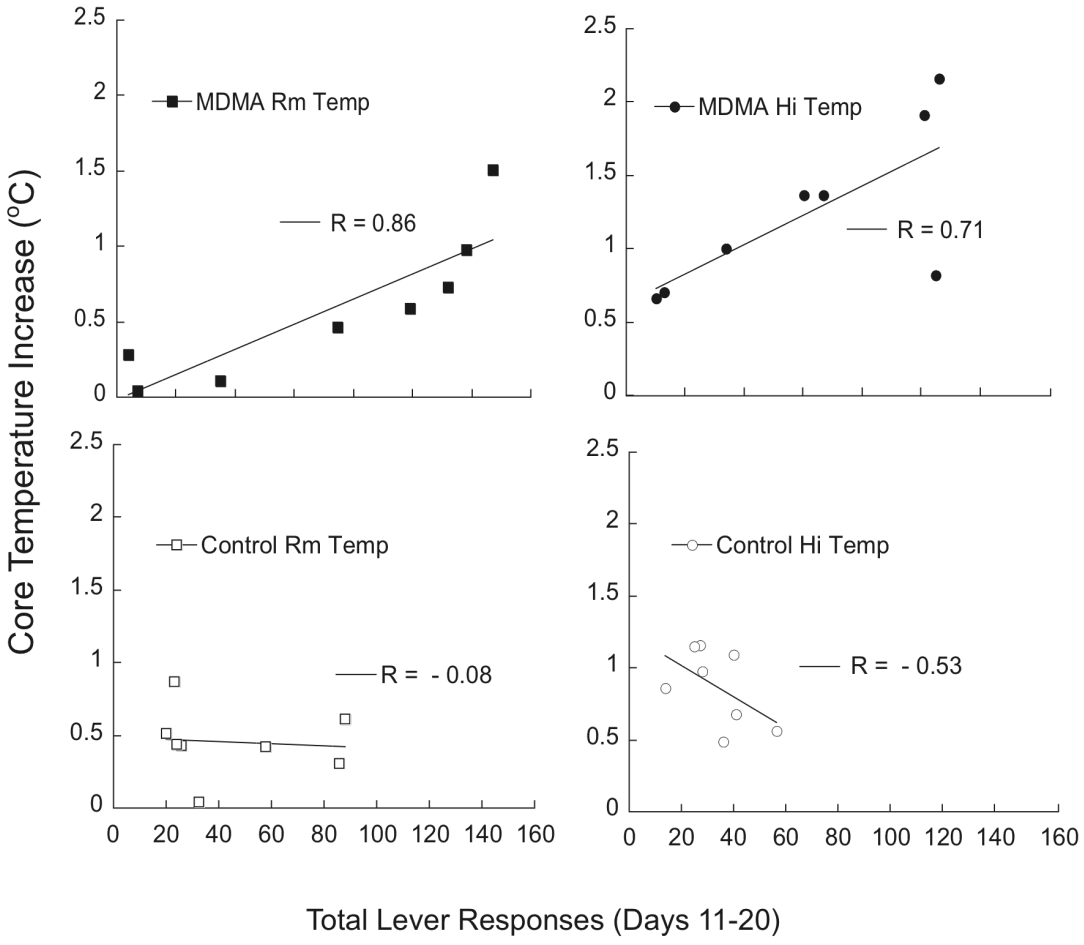


Figure 10: Self-Administration Sessions: Correlation between lever response totals and increased core temperature (core temperature before session minus core temperature post-session) during (A) Acquisition (Session Days 1-10) and (B) Maintenance (Session Days 11-20)

(A) MDMA High Temperature was the only group to show a significant positive correlation between lever responses and increased core temperature. **(B)** Significant positive correlations between lever responses and increased core temperature were revealed in both MDMA groups (High and Room Temperature) but not control groups.

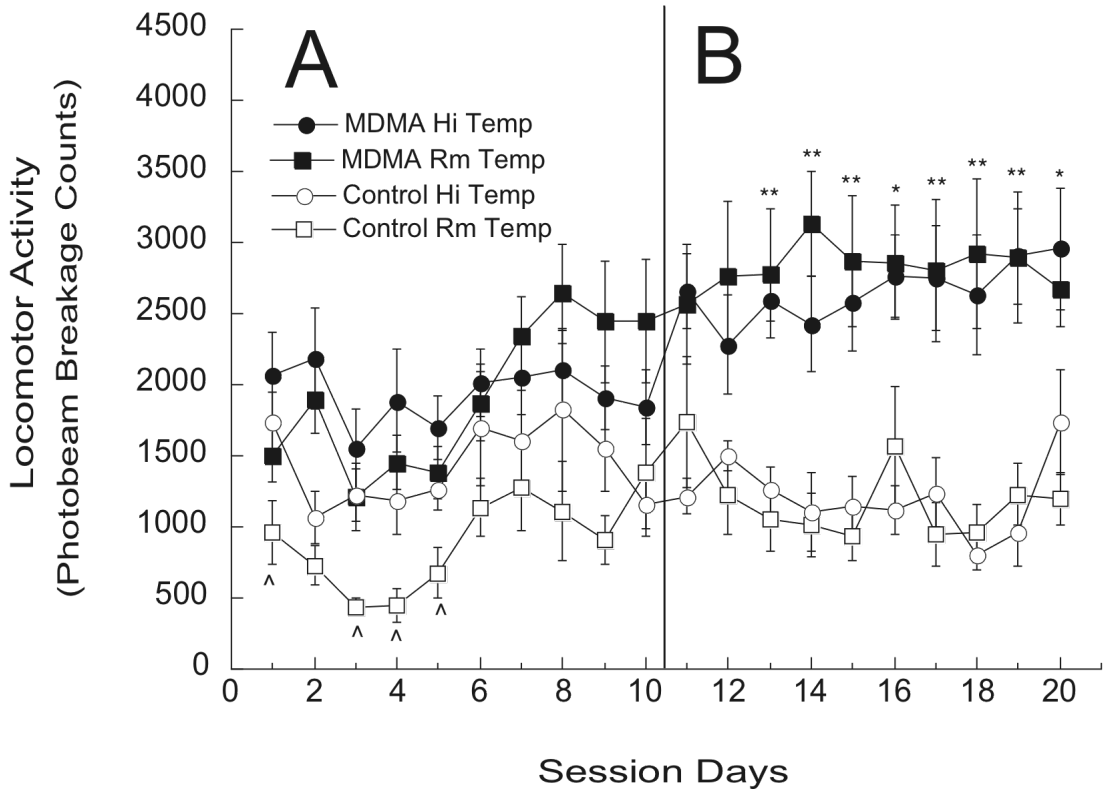


Figure 11: MDMA Self-Administration Sessions: Locomotor Activity

Mean daily locomotor activity units (+/- *SEM*) during **(A)** Acquisition (Session Days 1-10) and (B) Maintenance (Session Days 11-20). (A) MDMA groups had significantly greater overall locomotor activity than both Control groups, though activity in the Control High Temperature group was comparable to MDMA groups on a number of matched sessions. In a few matched sessions, activity levels in the Control High Temperature were higher than those observed Control Room Temperature (^ = significant difference between Control Room Temperature and Control High Temperature @ $p < 0.05$). **(B)** Ambient temperature did not influence locomotor activity in either condition. Activity levels in the MDMA groups were comparable and significantly greater than Controls (*, ** = both MDMA groups significantly greater than both Control groups @ $p < 0.05$ and 0.01, respectively).

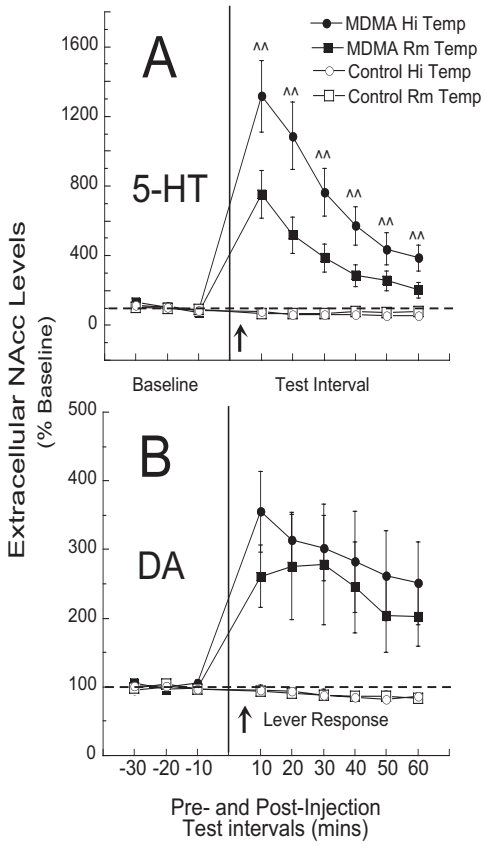


Figure 12: MDMA Challenge Test Session

Data represent mean (+/- *SEM*; expressed as Baseline mean %) (A) NAcc 5-HT, and (B) NAcc DA before (Baseline) and after a self-administered injection of MDMA (3.0 mg/kg) or saline (0.1 ml) in MDMA High Temperature (n=8), MDMA Room Temperature (n=8), Control High Temperature (n=8) and Control Room Temperature groups (n=8; same animals that had participated in the reported 20 daily self-administration sessions). **(A)** Both MDMA groups showed significant 5-HT enhancement from baseline levels, while Control groups showed no change in 5-HT. The magnitude of MDMA-induced 5-HT response was significantly greater in the MDMA High Temperature group compared to all other groups (^, ^^ = MDMA High Temp significantly greater than MDMA Room Temp @ $p < 0.01$, 0.05, respectively). **(B)** Both groups self-administering MDMA showed a significant increase in NAcc DA from baseline, and maintained higher levels of DA than Control groups after MDMA infusion. Control groups showed no significant changes in NAcc DA from baseline levels.

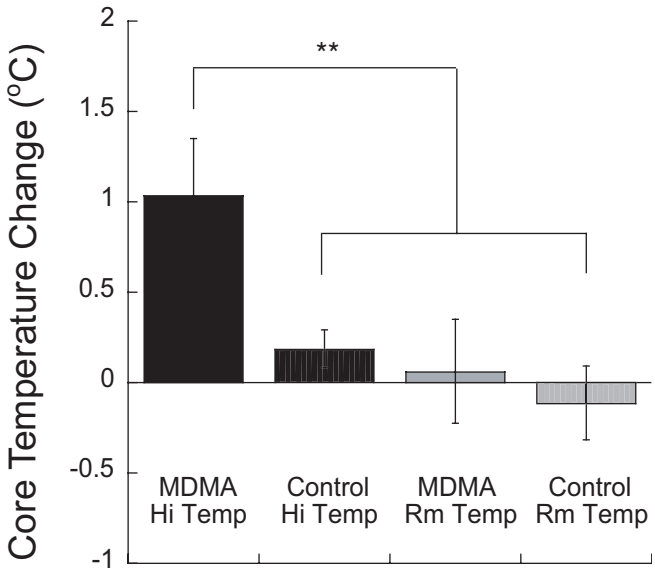


Figure 13: MDMA Challenge Test Session: Core Temperature Difference Scores

Data represent the mean (+/- *SEM*) of core temperature difference scores (After Session minus Before Session) Core temperature. The MDMA High Temperature group showed the greatest change in body temperature compared to all other groups on in response to MDMA (3.0 mg/kg/i.v.) (** = MDMA Hi Temp significantly greater increase in core temperature compared to all other conditions @ $p < 0.01$).

3-5. DISCUSSION

Findings from the present experiment indicate that the heated environment did not influence voluntary MDMA intake across the entire span of MDMA self-administration sessions. However, other behavioral and physiological changes recorded during self-administration sessions (e.g., activity and core temperature responses) were influenced by heat and MDMA exposure. During the first 10 self-administration sessions (e.g., Acquisition interval), activity and core temperature indices differed from those observed during the last 10 sessions (e.g., during Maintenance), indicative of experience-dependent effects. In addition, during the MDMA Challenge test, a self-administered MDMA injection (3.0 mg/kg, i.v.) produced a significantly greater NAcc 5-HT response when rats occupied a heated environment (32° C) than an identical injection administered under normal ambient temperature conditions (23° C). MDMA significantly increased NAcc DA levels, but statistical differences between thermal conditions were not detected. Control groups showed no significant variation from basal NAcc DA or 5-HT levels across the entire test period, confirming that thermal effects alone did not impact DA and 5-HT in this region.

Previous work has shown that reliable MDMA self-administration behavior is achieved in operant-trained animals over several sessions in which the MDMA unit dosage in the last 10 sessions (Maintenance = 0.5 mg/kg/inj) is half the dose of the first 10 sessions (Acquisition = 1.0 mg/kg/inj) (Daniela et al., 2004; Ratzenboeck et al., 2001; Schenk et al., 2003; Schenk et al., 2007). It should be noted that a minimum lever response criterion, as has been utilized in previous work (e.g., Daniela et al. 2004), was not

required in the present experiment. As a result, lever response rates shown here are lower in comparison due to inclusion of data from all animals in the experiment, not just those that attained high intake levels. In addition, Control groups (e.g., rats receiving saline infusions) showed the highest response rates during Acquisition. As indicated above, all animals in the experiment underwent food-reinforced operant training prior to surgical procedures. High rates of non-reinforced responding during initial Acquisition sessions are not unanticipated under the circumstances as this is a typical response pattern during extinction of food-reinforced training (Olds and Christenson, 1970). We have previously reported the same pattern of responding in Control animals under similar conditions (Reveron et al., 2006). Even though these non-reinforced responses were high for the first session, the number of lever presses dramatically decreased by the next session and continued at low levels for the remaining sessions (see Fig 8). Subsequently, during the Maintenance phase, MDMA-reinforced responding was significantly higher than Controls.

The present findings show that a heated environment does not increase the rate of MDMA intake and appears at odds with a previous study reporting a heat-induced increase in MDMA self-administration (Cornish et al., 2003). However, several methodological details differed between the present and the previous study. In the current study, MDMA self-administration at 32° C proceeded over 20 sessions. In the previous study, rats were tested during a single self-administration session at 30°C after MDMA self-administration responding had stabilized at room temperature (21°C) (though the number of MDMA self-administration sessions were not specified). It is not entirely surprising the different procedures would yield different outcomes and interpretations of the findings. However, in the present study and past work (Reveron et

al., 2006), we observed that MDMA-experienced rats increase MDMA intake over time. In addition, novelty has been shown to enhance rewarding effects of an environment (Bevins et al., 2002). Therefore, one possible explanation for enhanced responding reported in the previous study is that the combination of novel stimuli (e.g., higher temperature environment) and increasing MDMA experience may account for increased self-administration behavior during a heated test session. Future studies of this phenomenon could examine whether animals with stable MDMA self-administration rates while in a heated environment will alter response rates when placed in an operant environment set at room temperature. In that way, effects of novelty and drug experience may be addressed.

Progressive changes in core temperature responses during self-administration sessions suggest experience-dependent effects of heat and MDMA exposure. For instance, during the Acquisition phase (sessions 1-10), core temperature effects were not significantly altered in any of the experimental conditions. Yet, core temperature readings obtained during the Maintenance phase (sessions 11-20) showed significant enhancement in the MDMA High Temperature group compared to the MDMA and Control Room Temperature groups, but not when compared to the Control High Temperature group. Correlation analyses revealed that hyperthermic responses to MDMA administration were also progressively enhanced with experience. For example, during Acquisition, the number of MDMA-reinforced lever responses was positively correlated with increased core temperature in the MDMA High Temperature, but not the MDMA Room Temperature group. However, during Maintenance, the positive correlation between these factors was significant in both the MDMA High Temperature and Room Temperature groups. Taken together, these findings suggest that homeostatic

thermoregulatory responses become less effective under conditions of increased and/or repetitive exposure to MDMA and that a heated environment exacerbates MDMA-induced thermal dysregulation (Dafters, 1994; Green et al., 2004b; Sanchez et al., 2004).

In accordance with previous findings (Bankson and Cunningham, 2002; Gold and Koob, 1989; Spanos and Yamamoto, 1989), the current study showed locomotor activity significantly increased during MDMA self-administration in both the Room Temperature and High Temperature conditions. Since activity levels in both MDMA thermal conditions were comparable, the data indicate that MDMA-stimulated locomotor activity is unaffected by ambient temperature, as reported by others (Dafters, 1994; O'Shea et al., 2005). Locomotor activity increased as sessions proceeded, which is consistent with findings of drug-induced locomotor sensitization, which also been reported by numerous previous studies (Kalivas et al., 1998; Reveron et al., 2006; Spanos and Yamamoto, 1989). However, since MDMA intake also increased over time, increased activity may be attributable to the increase in MDMA dose rather than an exaggerated response to MDMA.

Elevation of synaptic 5-HT and DA is the primary mechanism of action by which MDMA exerts its major effects (Gough et al., 1991; Gudelsky and Nash, 1996; Gudelsky and Yamamoto, 2008; McCreary et al., 1999; Rudnick and Wall, 1992). Consistent with previous work, in the present study, both groups receiving MDMA had a robust enhancement of NAcc 5-HT and a significant, but less exacerbated release of DA (Baumann et al., 2008; Koch and Galloway, 1997; Kurling et al., 2008). In addition, 5-HT, but not DA, was significantly higher in the MDMA High Temperature condition compared to all other groups. These findings are in partial agreement with previous work

showing that MDMA-induced (2.5 and 5 mg/kg, i.p.) NAcc 5-HT responses were enhanced in an elevated temperature condition (30° C) compared to 5-HT responses in a lower temperature environment (20° C) (O'Shea et al., 2005). Also consistent with the present work, this study showed that ambient temperature did not influence locomotor activity, but the heated environment (30°C) enhanced MDMA-induced hyperthermia. Though the previous finding reporting enhanced levels of DA in a heated environment diverged from our statistical findings, a closer evaluation of the current data shows higher mean DA values at all intervals in the heat condition. This observation suggests that a larger sample size would yield significant effects of heat. However, since significant effects of temperature were observed in 5-HT responses with the present sample size, it is difficult to justify the use of additional animals to attain the statistical power needed to obtain another significant, though less dramatic effect. Still, there were also differences between our work and the previous studies that may account for our observation of a less robust differentiation between DA responses. For instance, the previous study utilized drug-naïve rats, while in the current study rats had extensive MDMA experience prior to dialysis testing. In addition, factors that influence the magnitude and duration of drug-induced neurochemical responses also varied, including route and mode of MDMA administration (Battaglia et al., 1988; O'Shea et al., 1998; Spanos and Yamamoto, 1989). Thus, it is possible that, in the previous study, the combination of higher temperature and differing experimental conditions may have affected DA neurotransmission to a greater extent than in the present report.

Neuroimaging studies have demonstrated reduced 5-HT transporter ligand binding and changes in the 5-HT_{2A} receptor in abstinent recreational users, indicative of depleted 5-HT levels (Cowan et al., 2003; Reneman et al., 2002; Semple et al., 1999). In addition,

clinical studies report memory impairment in current and abstinent Ecstasy users is common, and perhaps indicative of neural damage (Quednow et al., 2006; Zakzanis and Campbell, 2006) and decreased 5-HT function (Verkes et al., 2001). As reported here, high ambient temperatures increased MDMA-stimulated 5-HT release and hyperthermia, indicating a greater likelihood of MDMA-induced 5-HT attenuation after drug use in a heated environment. Since the MDMA dosages and intake examined in the present study were at low to moderate levels, these findings hold particular relevance for Ecstasy users whose drug use often occurs in hot, overcrowded environments. In addition, our findings indicate that progressive enhancing effects of MDMA on core temperature occurs in both normal and heated environmental conditions; hence repetitive use of low MDMA doses can result in MDMA-induced hyperthermic responses regardless of thermal conditions.

In conclusion, our findings indicate that ambient temperature does not change the reinforcing efficacy of MDMA, but enhances 5-HT efflux. For human users, these findings suggest that hot environments would not immediately initiate greater levels of MDMA-seeking or administration. However, the heat-induced increase in the magnitude of MDMA-stimulated 5-HT responses could lead to residual, prolonged 5-HT depletion; a condition that has been associated with escalation of drug intake in experienced Ecstasy users (Parrott, 2005). In addition, as 5-HT depletion has also been linked with severe effects on cognition (Quednow et al., 2006; Zakzanis and Campbell, 2006), mood and aggressive bias (Curran et al., 2004), the combination of heat and MDMA use poses an insidious threat, extending even to those who consider their drug use as "recreational".

CHAPTER 4: NOVEL APPARATUS AND METHOD FOR DRUG REINFORCEMENT

4-1. ABSTRACT

Animal models of reinforcement have proven to be useful in understanding the mechanisms underlying the neurobiology of addiction. Operant drug self-administration and conditioned place preference (CPP) procedures are expansively used in research to model various components of drug reinforcement, consumption, and addiction in humans. In this report, we combined traditional CPP and self-administration methods for a novel approach to studying drug reinforcement and addiction in rats. This new method provides more informative data than either method alone and improves upon some of the inherent drawbacks of CPP and self-administration procedures. In the short-term experiment, two groups of Sprague-Dawley rats self-administered cocaine (0.75 mg/kg, i.v.) or MDMA (0.5 mg/kg, i.v.) for four days in a distinctive environment. On alternate days, in a different environment, saline was available for self-infusion (0.1 ml, i.v.). Following self-administration/conditioning sessions, place preference was measured in the same apparatus. For the long-term experiment, the conditions and drug doses were the same except rats had access to cocaine for 8 days and saline for 8 days. In the short-term experiment, cocaine induced a positive CPP while MDMA and saline controls did not. Furthermore, drug lever responses were positively correlated with CPP scores for the cocaine long-term group only. This method was also sensitive in determining within-group differences of cocaine self-administration and CPP expression. These results demonstrate that this novel apparatus and approach is a valid animal model for the use of

studying drugs of abuse and had advantageous over traditional CPP and self-administration methods.

4-2. INTRODUCTION

Operant intravenous drug self-administration and place conditioning procedures are reliable and valid animal models for studying the neuropsychobiological basis for drug dependence and addiction in humans (Koob, 1995; Sanchis-Segura and Spanagel, 2006; Tzschentke, 2007). Both methods are widely used in pre-clinical drug abuse research and are able to measure the reinforcing properties of abused drugs (Bardo and Bevins, 2000). However, both methods have potential pitfalls in which the new apparatus and method presented in this paper will improve upon (Panlilio and Goldberg, 2007).

Place Conditioning Procedures are used to measure the subjective, reinforcing or aversive properties of natural rewards and drugs of abuse. Based on Pavlovian conditioning principles, the drug's reinforcing effects act as an unconditioned stimulus (UCS), are repeatedly paired with previously neutral environmental stimuli. After conditioning, the environment acts as a conditioned stimulus (CS) and can elicit approach or avoidance behaviors. The apparatus used in this paradigm consists of a conditioning box with two distinct environments differing in color, floor texture, lighting, or odor. After multiple days of conditioning, one compartment becomes associated with the drug, while the other compartment is linked to saline. On the test day, animals in a drug-free state are allowed access to both compartments. A conditioned place preference (CPP) is indicated by an increase in time spent in the drug-paired compartment and a conditioned place aversion (CPA) by a decrease in time spent in the drug-paired compartment (Tzschentke, 1998).

One large drawback of the CPP procedure is that the drug is administered non-contingently, which has different behavioral and neurochemical outcomes compared to drugs that are self-administered (Di Ciano et al., 1998; Miguens et al., 2008; Moolten and Kornetsky, 1990; Palamarchouk et al., 2009; Stefanski et al., 2007; Twining et al., 2009)). The differences can be attributed to the mode of drug intake that does not accurately model of the human experience. Additionally, CPP paradigms do not measure the progressive change in motivation to increase drug-intake, which is a key turning point in the switch from recreational drug use to uncontrollable drug addiction.

Operant drug self-administration procedures provide a good model for human drug consumption and method for studying the neurobiology of drug reinforcement (Panlilio et al., 2005). For the most part, drugs that are readily self-administered in animals have a high abuse potential in humans (Panlilio and Goldberg, 2007). There are various schedules of reinforcement (fixed, progressive, ratio, interval) and routes of drug administration (intravenous, intraventricular, intracranial, intragastric, oral) routinely used in these paradigms that can affect the number of infusions or rate of responding (Arnold and Roberts, 1997; Sanchis-Segura and Spanagel, 2006).

The operant self-administration method also has drawbacks. The rates of responding are used to infer reward value but can be affected by motor drug effects. The presence or absence of drug infusion-associated stimuli can also change the number of drug responses and extinction behaviors (Schindler et al., 2002). Another criticism of the operant self-administration method is that animals are pressing the lever for a drug because they were previously trained to lever press for food.

The novel apparatus and approach presented here, combines aspects of both methods to circumvent some of the inherent problems with traditional CPP and self-administration models. This model allows subjects to voluntarily self-administer drugs and then be tested in a drug free state. Furthermore, the ability to test conditioned place preference in a drug-free state bypasses the problem of testing reward while an animal is under the influence of the drug. This method could provide further information regarding this criticism that rats are lever pressing due food training because the CPP test scores could be correlated with the number of lever responses during conditioning (e.g., a strong positive correlation of lever responses and CPP scores would indicate the rat was indeed responding for the drug and not for food.)

To test the effectiveness of this apparatus and method, animals self-administered either cocaine (0.75mg/kg, i.v.) or MDMA (0.5 mg/kg, i.v.) and on alternate days, in a different environment, saline was available for self-infusion (0.1 ml, i.v.). The control group self-administered saline (0.1 ml, i.v.) in both compartments. The short-term groups had daily access to drug or saline for 8 consecutive conditioning sessions while the long-term groups had 16 consecutive conditioning sessions. On the final day, place preference measurements. Findings confirmed that this method is useful in studying the drug reinforcement in rats. Cocaine-induced conditioned place preference in the short-term experiment, and CPP was positively correlated with drug lever responses in the long-term experiment. Contrarily, MDMA or saline did not result in a CPP in this experiment. By combining traditional CPP and operant drug self-administration methods, more information is gathered and could potentially be more accurate in reflecting the abuse liability in humans.

4-3. MATERIALS AND METHODS

4-3-1. Animals

Male Sprague-Dawley rats (5 weeks old at start of experiment, Charles River Laboratories, Inc., Wilmington, MA) were in housed in clear cages in an animal colony with a 12:12 reverse light dark cycle. Rats were handled for 2 weeks prior to the start of the experiment. All protocols and procedures were in accordance with the Guide for the Care and Use of Laboratory Animals (U.S. Public Health Service, National Institute of Health) and the Institutional Animal Care and Use Committee (IACUC) at the University of Texas at Austin.

4-3-2. Apparatus

The apparatus consisted of two distinct operant chambers (28 x 22 x 21 cm) (Med Associates, St. Albans, VT) with an additional constructed alley between (21 x 25 x 25 cm), which were separated with removable panels to allow access during the CPP experiment and confinement during self-administration. The tops of the operant chambers had an opening to allow for a tether system to enter the chamber. The alley consisted of two black walls with white stripes and two metal walls, a clear Plexiglas top, and a white Plexiglas floor. The two main chambers had a steel grid rod floor consisting of 3/16" (4.8 mm) rods, placed on 5/8" (16 mm) centers. In order to create unique, distinguishable environments, the operant chambers (top, front, and back walls) were covered with either white or black material and a cotton ball scented with either rose or cinnamon was placed on the removable tray under the retractable lever. A house light within both chambers remained on during all sessions. For self-administration sessions, retractable levers were available at opposite ends of the two operant chambers with a

stimulus light located directly above. The rats' catheters were connected to a spring-covered tubing (Plastics One, Roanoke, VA) that attached to a drug swivel mounted on a balancing arm. Upon pressing the lever, the stimulus light was turned on and a motor-driven syringe pump (Razel, St. Albans, VT) infused 0.1 ml of saline or drug solution for 6 sec into the animal's catheter. To measure animal locomotion, three pairs of infrared photodetectors embedded in the walls of each compartment, detected photobeam breakages. During CPP baseline and preference test, a video camera mounted above the apparatus recorded entrance and exits into each chamber. A blind experimenter viewing the recordings determined the amount of time the animals spent in each compartment. A Med Pentium 100 MHZ computer equipped with Med-PC software monitored and recorded locomotor activity and lever responses.

4-3-3. Food Training

A fixed ratio (FR1) schedule of reinforcement with food reward (45 mg sucrose pellets; Bio-Serv, Frenchtown, NJ) was used to train rats to lever press. Food training lasted for approximately 8 days of 10 min/day operant sessions. Animals were food restricted (approximately 6 g of laboratory rat chow per day, adjusted to maintain weight) until lever responding was acquired.

4-3-4. Surgical Procedures

To enable intravenous drug or saline delivery, rats underwent a jugular catheterization procedure as previously described (Feduccia 2008). For the surgical procedure, rats were anesthetized with 2.5% isoflurane (VetEquip, Pleasanton, CA) vaporized in oxygen at a flow rate of 0.8 L/min. One dose of Rimadyl (5 mg/kg, s.c.) was administered after the

surgery for prophylactic pain relief. To maintain patency, jugular catheters were flushed daily with 0.1 ml of 0.9% saline containing 1 U/ml heparin and 67 mg/ml Timentin. After one week, daily catheter flushing continued with only 0.1 ml of 0.9% saline throughout the duration of the experiment.

4-3-5. MDMA and Cocaine

(+/-) 3,4-methylenedioxymethamphetamine HCl (MDMA) (NIDA Drug Inventory Supply and Control Program; RTI International, Research Triangle Park, NC) and Cocaine (RTI International, Research Triangle Park, NC) was used in this experiment. MDMA and Cocaine was dissolved in isotonic saline solution (0.9 %) in the appropriate dose concentrations according to the weights of the animals.

Table 2: Experimental Groups

Group	# of Drug-pairings	# of Saline-pairings	n size
Control short-term	0	8	10
MDMA short-term	4	4	7
Cocaine Short-term	4	4	8
Control long-term	0	16	9
MDMA long-term	8	8	8
Cocaine long-term	8	8	8

4-3-6. Experimental Procedures (CPP and Self-Administration)

Baseline Preference

The conditioned place preference experiment started with 2 days of baseline measurement, with animals having access to both compartments (lever unavailable) of the apparatus. After a 5-minute habituation period, rats were allowed to habituate to apparatus and then the duration of time spent in each compartment (white vs. black) was recorded for the following 15 minutes.

Self-administration/Conditioning Sessions

Self-administration sessions began on next day and lasted for 8 days (short-term groups) or 16 days (long-term group). During self-administration sessions, the house light illuminated and the lever was made available for 1 hour. A progressive ratio (PR) schedule of reinforcement allowed for delivery of drug (MDMA or Cocaine) and on alternate days saline. Each infusion resulted in the lever retracting the stimulus light illuminating. The following progression of response requirements was utilized: 1, 3, 6, 10, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268. Control groups self-administered saline (0.1 ml/inj) with a PR schedule through out all conditioning sessions.

Preference Test Session

After the last conditioning session, animals were tested for place preference. For the Preference Test session, the lever was retracted and rats were allowed access to both compartments for 20 minutes. After a 5-minute habituation period, the time spent in each compartment was measured for 15 minutes.

4-3-7. Statistical Analysis

Place Conditioning

Difference scores at baseline and test were calculated by subtracting the number of seconds spent the saline-paired side from the number of seconds spent on the drug-paired side. For the control group, difference scores were determined by subtracting time (seconds) in saline-paired minus time (seconds) in the other saline-paired compartment. Baseline was calculated by averaging the difference scores from baseline days 1 and 2. CPP's were determined by comparing the difference scores of drug groups with the difference scores of the Control group using a two-way repeated measures analysis of variance (Treatment x Time [Baseline, Post-test]), with separate analysis for short- and long-term groups. Paired samples t-test was utilized in the locomotor activity (e.g., photobeam breakages) analysis of drug-paired versus saline-paired sides on baseline and post-test days. Pearson's Correlation analyses determined relationships between the total number of drug lever responses and CPP post-test scores. Fisher's Least Significant Differences (LSD) post hoc analyses were used when main effects (at least $p < 0.05$) were found in the initial analyses.

Conditioning/Operant Sessions

Independent samples t-test was utilized in analysis of daily lever responses, daily infusions, and daily locomotor activity (e.g., photobeam breakages) to compare short- and long-term groups.

Low vs. High Cocaine Responders in Long-term Experiment

Since there was a positive correlation between total drug lever responses and post-test CPP scores in the Cocaine long-term group, rats were divided into 2 groups: Cocaine High Responders and Cocaine Low Responders. Rats with more than 100 drug lever responses were placed in the High Responders group; rats with less than 100 drug lever responses were placed into the Low Responders group. An independent samples t-test was utilized in the analysis of place preference, drug lever responses, drug infusions, and locomotor activity. As with all statistical analyses, Fisher's Least Significant differences were performed during post hoc analyses.

4-4. RESULTS

4-4-1. Place Conditioning

Place Conditioning short and long-term

Difference scores at baseline and test were calculated by subtracting the number of seconds spent the saline-paired side from the number of seconds spent on the drug-paired side. Baseline was calculated by averaging the difference scores from baseline days 1 and 2. **Short-term Groups:** A two-way repeated measures ANOVA (Treatment x Time) showed significant Time x Treatment Interactions [$F(2,22) = 4.187$, $p < 0.05$], but no significant Time [$F(1,22) = 0.056$, *n.s.*] or Group [$F(2,22) = 3.07$, *n.s.*] effects. Post Hoc tests revealed no significant differences between all groups' baseline difference scores. The Cocaine group had significantly higher post-test scores compared to the Control group ($p < 0.05$) and MDMA group ($p < 0.01$) post-test scores (see Fig 14A).

The Cocaine group also showed a significant increase in post-test score compared to baseline ($p < 0.05$). **Long-term Groups:** A two-way repeated measures ANOVA (Treatment x Time) showed significant Time x Treatment Interactions [$F(2,22) = 1.319$, *n.s.*], but no significant Time [$F(1,22) = 1.112$, *n.s.*] or Group [$F(2,22) = 0.957$, *n.s.*] effects (see Fig 14B).

Correlation Analysis of drug lever responses and CPP scores

A significant correlation between total drug lever responses and post-test CPP % scores was determined for Cocaine long-term group [$r = 0.711$, $p < 0.05$] but not Cocaine short-term [$r = 0.112$, *n.s.*], MDMA short-term [$r = - 0.269$, *n.s.*], or MDMA long-term [$r = 0.217$, *n.s.*] (See Fig 15).

4-4-2. Conditioning/Operant Sessions

Lever Responses

An independent samples t-test of sessions' mean lever responses between short- and long-term groups: Cocaine [$t(15) = 0.638$, *n.s.*], MDMA [$t(13) = 1.301$, *n.s.*], and Control [$t(16) = 1.430$, *n.s.*].

Infusions

An independent samples t-test of sessions' mean infusions between short- and long-term groups: Cocaine [$t(15) = 0.812$, *n.s.*], MDMA [$t(13) = 1.311$, *n.s.*], and Control [$t(16) = 1.230$, *n.s.*].

Locomotor Activity

An independent samples t-test of sessions' mean locomotor activity between short- and long-term groups: Cocaine [$t(15) = -0.135$, *n.s.*], MDMA [$t(13) = 3.031$, $p < 0.01$], and Control [$t(16) = -0.516$, *n.s.*].

4-4-3. Low vs. High Cocaine Responders in long-term experiment

Place Conditioning Low vs. High Cocaine Responders

Place Conditioning

An independent samples t-test showed High Responders having significantly greater post-test CPP scores compared to Low Responders [$t(7) = 9.592$, $p < 0.05$] (See Fig 18).

Conditioning/Operant Drug Sessions for Low vs. High Cocaine Responders

Lever Responses

An independent samples t-test showed significant differences between High and Low Responders sessions' mean drug lever responses [$t(7) = 6.299$, $p < 0.001$] (See Fig 19).

Infusions

An independent samples t-test showed significant differences between High and Low Responders of sessions' mean drug infusions [$t(7) = 6.473$, $p < 0.001$] (See Fig 19).

Locomotor activity

An independent samples t-test showed significant differences between High and Low Responders of sessions' mean drug locomotor activity counts [$t(7) = 3.112$, $p < 0.05$] (See Fig 20).

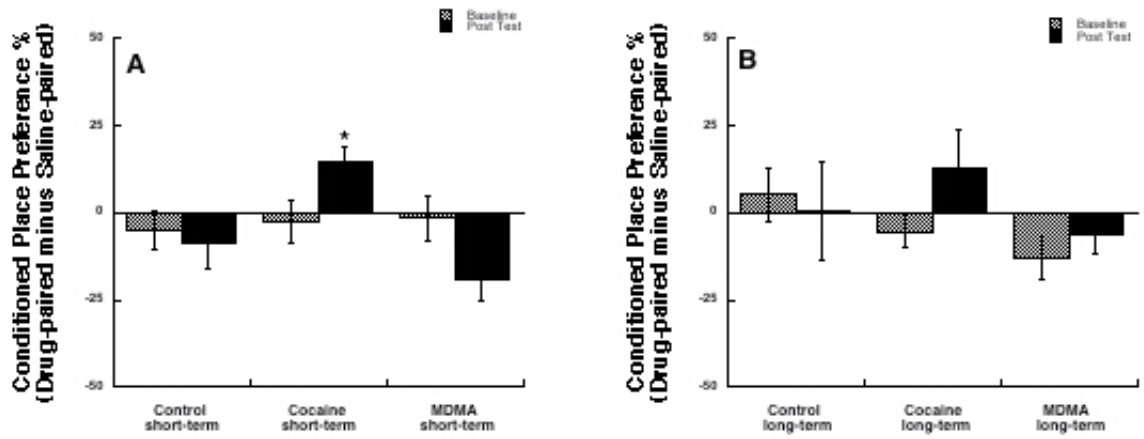
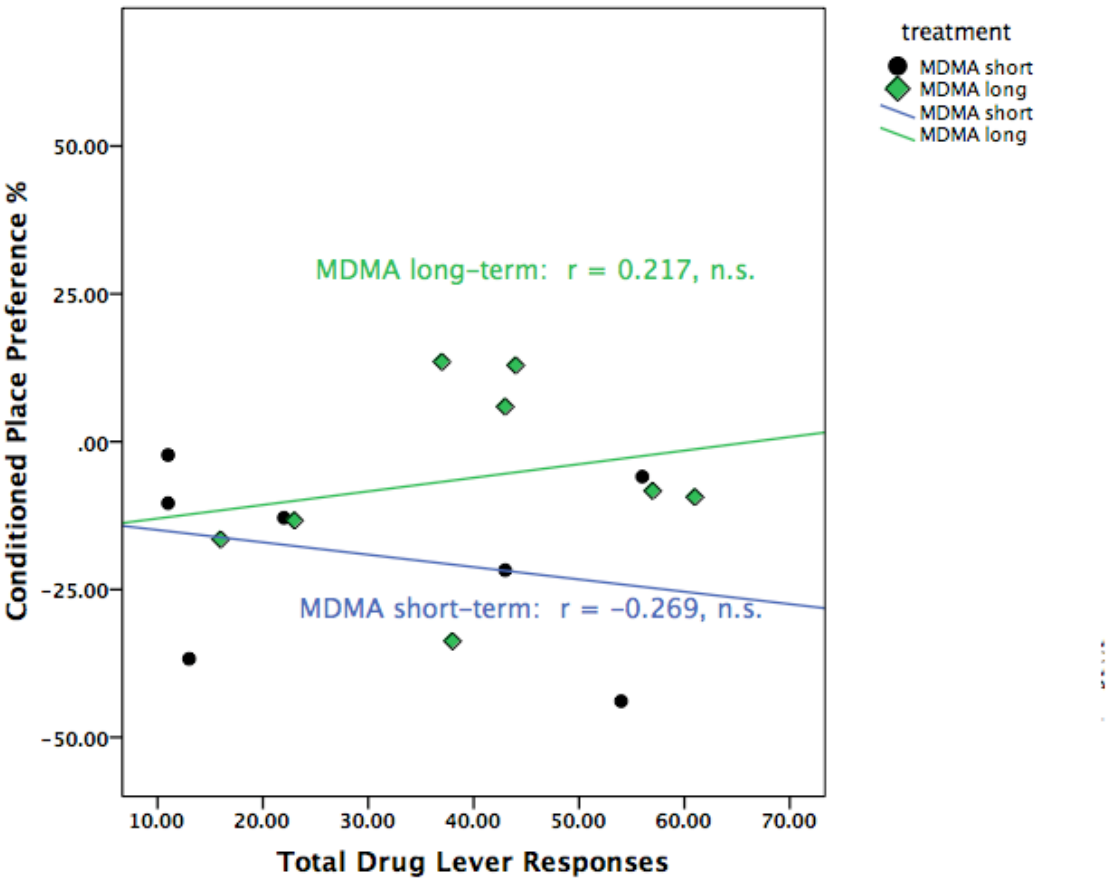


Figure 14: Place Conditioning: (A) Short-term groups (B) Long-term groups

Data are represented as mean (+/- *SEM*) difference scores: percent time spent in the drug-paired compartment minus the saline-paired compartment. No significant differences between all groups' baseline difference scores. **(A)** Cocaine short-term group showed a significant conditioned place preference after conditioning (* = $p < 0.05$ compared to Control group). **(B)** No significant differences at baseline or post-test detected for the long-term groups.


treatment
MDMA short
MDMA long
MDMA short
MDMA long
MDMA long-term: r = 0.217, n.s.
MDMA short-term: r = -0.269, n.s.
Conditioned Place Preference %
50.00
25.00
.00
-25.00
-50.00
10.00
20.00
30.00
40.00
50.00
60.00
70.00
Total Drug Lever Responses

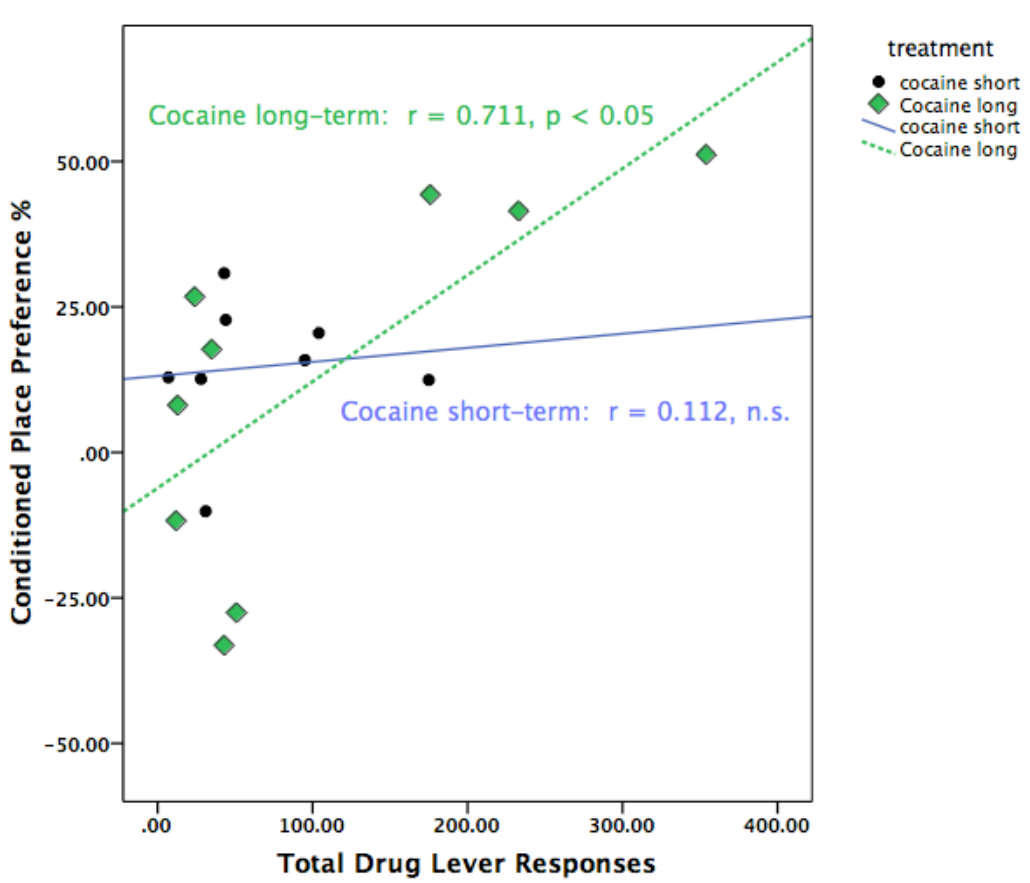
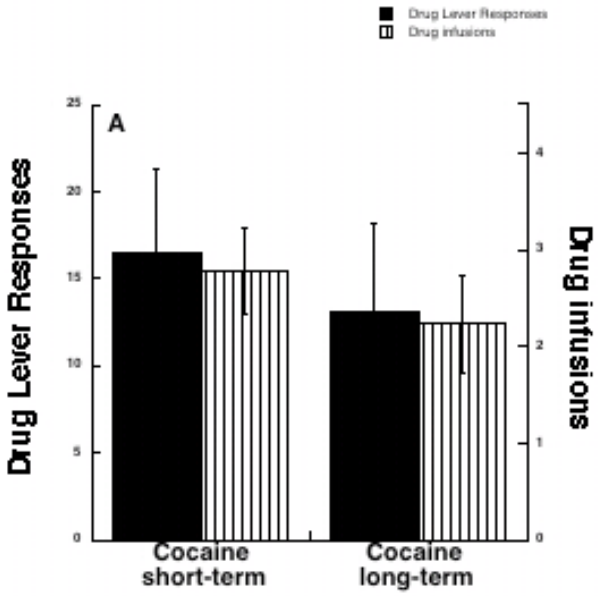
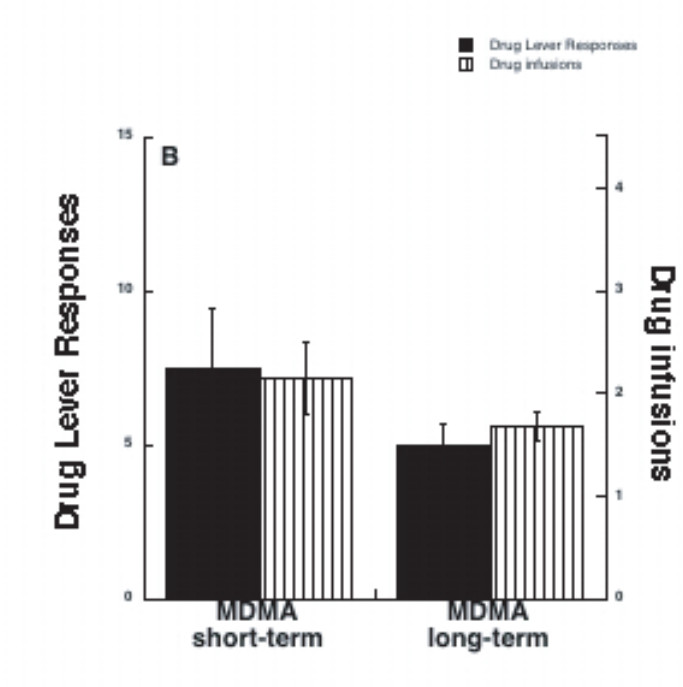


Figure 15: Drug Self-Administration Sessions: Correlation between total drug lever responses and CPP difference scores (% time drug-paired minus % time saline-paired) during Post-test

Cocaine long-term group was the only group to show a significant positive correlation between total drug lever responses and Post-test CPP scores ($p < 0.05$).


Drug Lever Responses
Drug infusions
A
Drug Lever Responses
Drug infusions
25
20
15
10
5
0
4
3
2
1
0
Cocaine
short-term
Cocaine
long-term


Drug Lever Responses
Drug infusions
B
Drug Lever Responses
Drug infusions
15
10
5
0
4
3
2
1
0
MDMA
short-term
MDMA
long-term

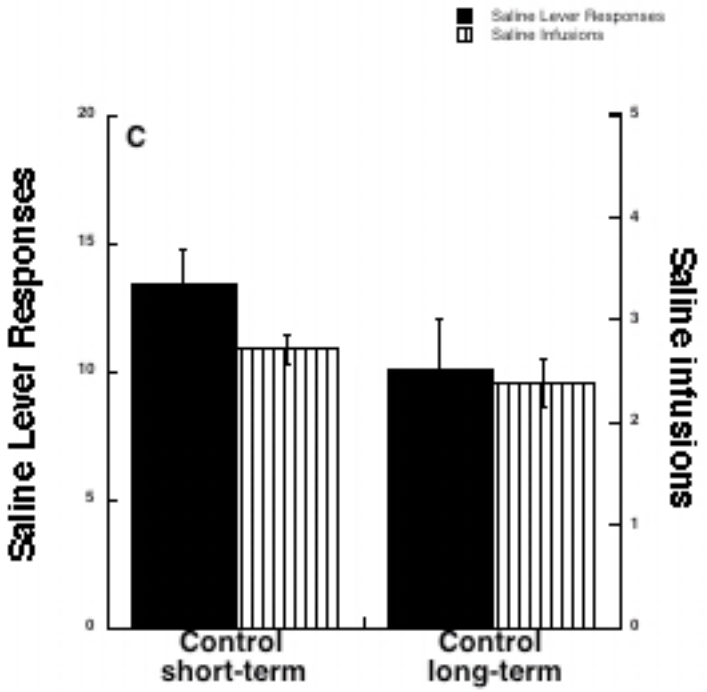
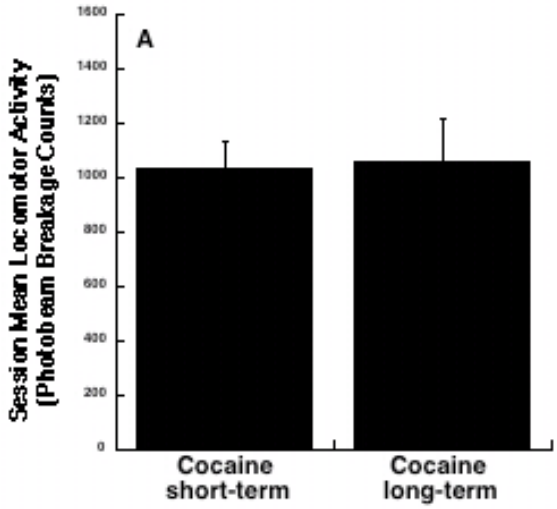
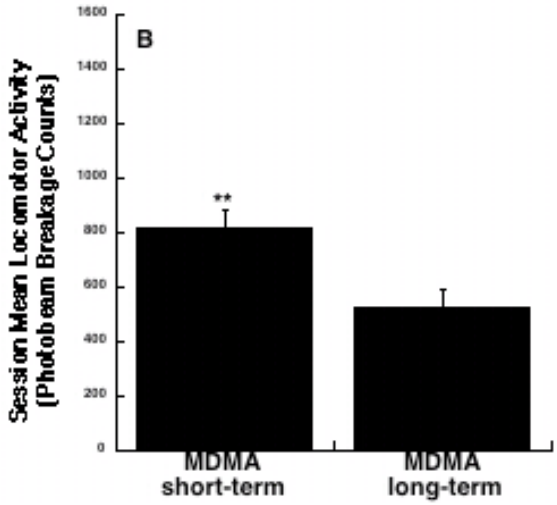


Figure 16: Self-Administration Sessions: Lever responses and infusions for (A) Cocaine, (B) MDMA, and (C) Control

The left y-axis represents mean (+/- *SEM*) daily lever responses and the right y-axis represents mean (+/- SEM) daily infusions. There were no differences between short- and long-term lever responses or infusions for any group.


A
Session Mean Locomotor Activity
(Photobeam Breakage Counts)
1600
1400
1200
1000
800
600
400
200
0
Cocaine
short-term
Cocaine
long-term


B
Session Mean Locomotor Activity
(Photobeam Breakage Counts)
1600
1400
1200
1000
800
600
400
200
0
**
MDMA
short-term
MDMA
long-term

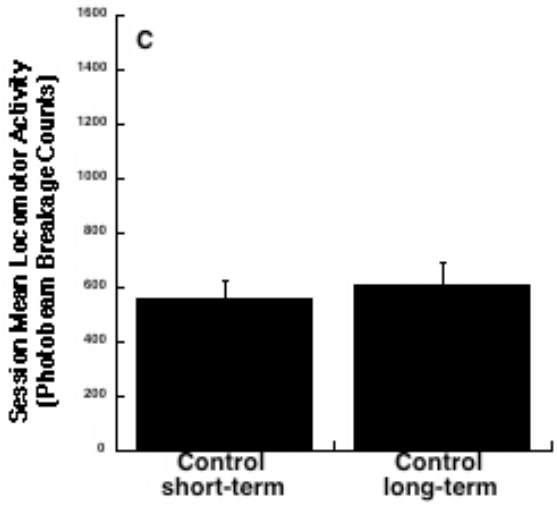


Figure 17: Self-Administration Sessions: Locomotor activity for (A) Cocaine, (B) MDMA, and (C) Control

Bars represent mean daily locomotor activity units (+/- *SEM*) during drug self-administration sessions. MDMA short-term group had greater locomotor activity counts than the MDMA long-term group (** = $p < 0.01$). There were no differences between short- and long-term locomotor activity for Cocaine and Control groups.

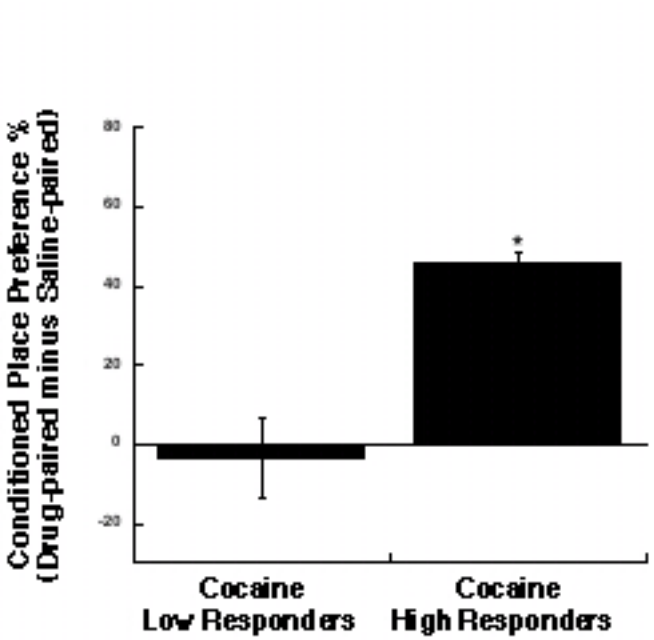


Figure 18: Low vs. High Cocaine Responders Place Conditioning

Data are represented as mean (+/- *SEM*) difference scores: percent time spent in the drug-paired compartment minus the saline-paired compartment for the Cocaine long-term group divided into Cocaine High Responders and Cocaine Low Responders. High Responders showed significantly greater post-test CPP scores compared to Low Responders (* = $p < 0.05$).

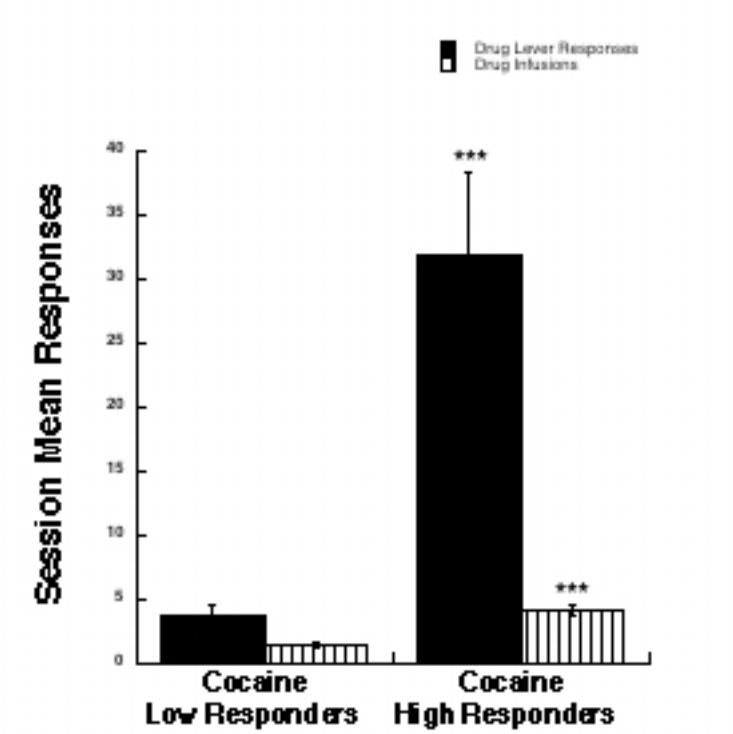


Figure 19: Low vs. High Cocaine Responders Self-Administration Sessions: Lever responses and infusions

Mean (+/- *SEM*) daily lever responses and mean (+/- *SEM*) daily drug infusions during drug sessions. **(A)** Cocaine High Responders had more drug lever responses and drug infusions compared to Cocaine Low Responders (*** = $p < 0.001$).

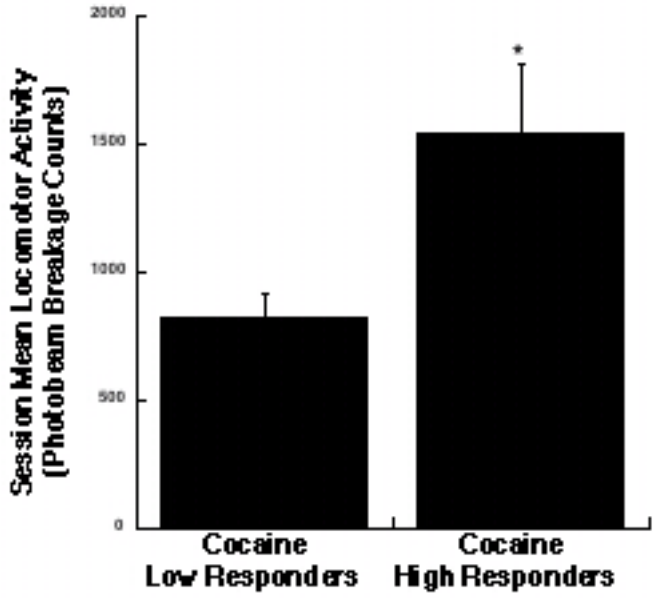


Figure 20: Low vs. High Cocaine Responders Self-Administration Sessions: Locomotor activity

Bars represent mean daily locomotor activity units (+/- *SEM*) during drug self-administration sessions. Cocaine High Responders had more drug lever responses and drug infusions compared to Cocaine Low Responders (* = $p < 0.05$).

4-5. DISCUSSION

Findings from these experiments demonstrate that this novel apparatus and method can be used to measure the reinforcing properties of drugs of abuse by combining traditional place conditioning and operant self-administration procedures. Furthermore, this design provides additional information by allowing the animal voluntary access to the drug and place preference testing in a drug-free state. In the short-term experiment, cocaine self-administration resulted in a conditioned place preference, while MDMA or saline did not. In the long-term experiment, no groups showed a significant CPP. There was no overall difference in daily drug lever responses or drug infusions between Cocaine and MDMA short- and long-term groups.

Many factors can influence the magnitude of CPP or CPA, including drug, dose, route of administration, and the number of drug-context pairings (Cunningham et al., 1999; Ettenberg et al., 1999; Mayer and Parker, 1993). Most drugs cause a dose dependent conditioned place preference in rats and other non-drug factors may be crucial to the development of the CPP (Gardner, 2000). These results support previous reports of drug reinforcement models. Cocaine is known to be readily self-administered by rats and produce a robust conditioned place preference at a number of doses and routes of administration (Liu et al., 2005; O'Dell et al., 1996; Tzschentke, 2007). A meta-analysis of cocaine CPP by Bardo *et. al.*, showed no dose-CPP effect relationship meaning there was no significant differences between the strength of CPP produced by a range of cocaine doses (0.1 – 10 mg/kg, i.v., s.c., and i.p. combined) included in the analysis (Bardo et al., 1995). However the authors note, that other studies have shown IP cocaine

administration to produce a monotonic ascending dose-effect curve and IV administration to produce an inverted U-shaped dose-effect curve (Nomikos and Spyraki, 1988). Separate analysis of each route of administration by Bardo *et. al.*, also failed to show a dose-effect response for cocaine CPP (Bardo et al., 1995). On the contrary, MDMA self-administration develops more gradually and MDMA-induced CPP occurs in a dose response manner, with optimal doses ranging from 2.0 – 10.0 mg/kg (Bilsky et al., 1990; Marona-Lewicka et al., 1996). Furthermore, other non-drug factors such as housing or auditory stimuli can affect MDMA reward efficacy (Feduccia and Duvauchelle, 2008; Meyer et al., 2002). The rats in this experiment self-administered approximately 1.0 mg/kg MDMA/session, which may have been too low of a dose for MDMA-induced place preference.

A strong positive relationship between drug lever responses and post-test CPP scores was determined only for the Cocaine long-term group. On the other hand, the short-term Cocaine group's positive CPP scores were not correlated with drug lever responses, with all rats showing approximately the same increase in preference irrespective of drug responses. By averaging the groups' scores, no CPP was detected for the long-term Cocaine group due to the large within group variance. These results suggest that place conditioning and self-administration are related but are not isomorphic measures of reward.

An interesting finding from this study is the divergence of two subpopulations of rats in the long-term cocaine experiment. Across the 8 drug sessions, the Cocaine High Responders (HRs) had an average of 254 total drug lever responses and a post-test CPP score of 46% while the Cocaine Low Responders (LRs) had an average of only 30 total

drug lever responses and a post-test CPP score of – 3%. HRs also had greater locomotor activity, drug lever responses, and drug infusions than LRs during drug sessions. The higher intake of cocaine by the high responders may partially explain the huge increase in CPP, but is likely reflecting a more robust change in reinforcement of the drug. As sessions progressed, the Cocaine High Responders showed greater locomotor activity counts than the Cocaine Low Responders. The enhanced activity is likely due to sensitization to the behavioral effects of cocaine, which is a common and thoroughly documented phenomenon that occurs with repeated psychostimulant exposures (Bjijou et al., 1996; Kalivas and Duffy, 1990; Vanderschuren and Kalivas, 2000).

Other studies have also reported the emergence of subpopulations in CPP experiments, based on time spent in the drug-paired environment on test-day (Adams et al., 2001; Daza-Losada et al., 2007). By averaging group scores, the differences between 'CPP expressing' and 'CPP non-expressing' rats are masked and conclusions are based on group means. A recent study addressed this problem by developing a criteria for dividing rats into subgroups based on CPP expression and showed this analysis to be more effective in indentifying pharmacological blockade of CPP in the subgroups (Dela Cruz et al., 2009). The method presented in this paper is also useful in determining subpopulations of CPP expression, and provides further information about these subgroups based on their rates of responding and motivation to acquire the drug.

The phenomenon of high and low responders is reported in several different areas of the literature. In a drug-free state, high responders will reliably prefer a novel environment and show an increase in locomotor activity when placed in a novel environment compared to a familiar one (Dellu et al., 1996). Furthermore, high responders have

greater locomotor activity after psychostimulant treatments and also have higher rates of responding than low responders (Kabbaj, 2006; Piazza et al., 1989; Piazza et al., 2000). Differences between HRs and LRs rats are attributed to varying sensitivity of the hypothalamo-pituitary-adrenal (HPA) axis, a key mediator of the stress response. When placed in a novel environment, HRs show larger elevations in plasma corticosterone levels that remains high for a longer duration compared to LRs (Kabbaj et al., 2000; Piazza and Le Moal, 1998; Piazza et al., 1991a). This differences may be attributed to a dysregulation of negative feedback to the HPA axis since HRs have less glucocorticoid receptors and lower corticosterone binding in the hippocampus compared to LRs (Kabbaj et al., 2000; Kabbaj et al., 2007). Furthermore, at baseline HRs have elevated levels of DA in the NAcc and lower DA and 5-HT levels in the prefrontal cortex (Hooks et al., 1992; Piazza et al., 1991b; Verheij et al., 2008). HRs also have lower concentrations of D2 receptors in the NAcc and striatum than LRs (Hooks et al., 1994; Kabbaj, 2004). Taken together, these variations in the HPA axis and dopaminergic systems can at least partially explain the behavioral differences between high and low responders.

Two previously published studies have reported a dissociation between CPP and self-administration. Bardo *et al*. first tested amphetamine in a 2-day CPP procedure followed by a daily amphetamine self-administration experiment (FR and PR schedules of reinforcement) and reported no correlation between CPP scores and rate of self-administration (Bardo et al., 1999). In a different study, rats self-administered cocaine for 6 or 29 sessions and then rats underwent a CPP test in a different testing chamber with 4 cocaine pairings (experimentally administered) and 4 saline pairings. Additionally, a cocaine naïve group was tested in the CPP procedures. A similar threshold dose of cocaine (0.4 mg/kg i.v.) induced CPP in both cocaine-experienced

groups (6 SA sessions and 29 SA sessions), while the cocaine naïve group needed a higher dose (0.8 mg/kg i.v.) to attain CPP. Even though the 29 SA session group self-administered more drug and showed differences in cocaine reinstatement, there was no overall group differences in time spent in the drug-paired compartment during the CPP test (Deroche et al., 1999).

These results also bring into question how valid is a short-term CPP procedure in modeling addiction behaviors in humans since we see such a large shift between the cocaine short- and long-term experiments. Short-term exposure is likely reflecting the acute reinforcing properties of the drug typically seen with recreational drug use while the escalation of drug use by the Cocaine High Responders in the long-term experiment may be modeling addiction processes. It is important to differentiate between what place conditioning and self-administration methods are modeling: reinforcement, reward, or addiction?

The new apparatus and approach permits animals to voluntarily self-administer drugs of abuse and allows for conditioned preference or aversion measurements. This method provides both direct and indirect information about the positive (reinforcement) and negative effects of drugs, and has the ability to test drug effects while the animal is in a drug-free state. Additionally, this is a more time and cost effective approach and gives more informative results compared to doing CPP and self-administration as separate experiments. Countless experimental designs could utilize these procedures to test a variety of addictive drugs and potential pharmacological treatments for addiction. The apparatus would be useful in determining relationships between voluntary drug intake behavior and experience-dependent changes in self-administration with changes in

conditioned drug effects. Furthermore, it could be used to investigate the influence of specific sensory stimuli on drug-seeking, reinstatement and spontaneous recovery. In conclusion, these methods can be used to test drug reinforcement and provides more information than CPP or self-administration alone and has great potential for future use.

CHAPTER 5: SUMMARY OF FINDINGS AND FUTURE DIRECTIONS

The overall findings for this dissertation:

1) Low doses of MDMA in the presence of white noise resulted in a significant place preference, while MDMA + Music and MDMA Alone did not.

2) Non-drug factors, such as auditory stimuli, play a critical role in the rewarding value of MDMA.

3) MDMA + Music showed the greatest increase in NAcc DA and 5-HT levels compared to MDMA Alone, and MDMA + Noise.

4) MDMA and music have a synergistic effect on monoamine levels, indicating that both work on common neural networks.

5) Locomotor activity enhanced on dialysis test only for MDMA + Music group.

6) There is no difference in long-term MDMA self-administration rates or locomotor activity counts between normal and high environmental temperatures (23° or 32°C).

7) MDMA significantly increased DA and 5-HT from baseline in both thermal conditions.

8) In the heated environment, MDMA-stimulated 5-HT response was greater than at room temperature, but there was no difference in DA responses between the two thermal conditions.

9) Combining traditional CPP and self-administration procedures offers a novel approach to studying drug reinforcement.

10) Cocaine produced a positive CPP in the short-term experiment but not in the long-term experiment. Only the Cocaine long-term group showed a significant correlation between total lever responses and post-test CPP % scores.

11) MDMA and saline control groups did not show a CPP in the short- or long-term experiments.

12) Rats in the long-term Cocaine group divided into 2 subpopulations: Low Cocaine Responders and High Cocaine Responders. High Cocaine Responders had greater lever responses, drug infusions, locomotor activity, and CPP post-test scores compared to Low Responders.

The studies presented in chapter 2 and 3 of this dissertation contribute significant, unique findings to the MDMA literature. Auditory stimuli and increased ambient temperature are often present in the environments that MDMA is taken and more research is needed to understand how these factors influence MDMA consumption, reinforcement, and neurotoxicity. Future directions would be to investigate the exact mechanisms and neural substrates underlying the effects seen in these studies. Also, experiments to understand how other sensory stimuli, such a visual and somatosensory, modify MDMA drug effects would contribute significantly to the literature. Another research avenue would involve testing the neurotoxic effects of MDMA in different environmental contexts and quantifying the potential mechanisms.

Chapter 4 presented a novel approach that can be used to study drug reinforcement and reward in animal models. The goal of the experiments was to test the new apparatus and method and provide evidence of the value for its future use. In addition to CPP and self-administration, several experiments could potentially use this design; namely, drug-seeking, reinstatement, and spontaneous recovery studies. Future aims could probe more deeply to understand to neural substrates and circuitry involved in place preference and the escalation of drug self-administration.

CHAPTER 6: CONCLUSIONS

There is a large body of work that has investigated large doses of MDMA in rodents, however, very few studies have focused on the rewarding properties of the drug at recreational doses. MDMA has not been shown to be as potent of a reinforcer as stimulant drugs, such as cocaine or methamphetamine, because self-administration response rates and speed of acquisition are lower (Reveron et al., 2006; Schenk et al., 2003). This may give human Ecstasy users the impression that the drug is not addictive and safe to use, but at this time there is insufficient research on the long-term effects of MDMA in humans. Furthermore, a growing body of evidence suggests that environmental stimuli can have profound effects on the pharmacological actions of MDMA in the brain (Feduccia and Duvauchelle, 2008; Parrott et al., 2008).

Since young adults at clubs or raves often take Ecstasy, the first aim of the dissertation was to determine how auditory stimuli (music, white noise, or no additional sound) impact MDMA-induced CPP, self-administration, and NAcc DA and 5-HT responses. These experiments demonstrated that low doses of MDMA in the presence of white noise resulted in a significant place preference, while MDMA + Music and MDMA Alone did not. Although not significant, it should be noted that MDMA in combination music produced an increase in preference for the drug-paired environment and with additional conditioning sessions or a slight change in the auditory stimuli this effect may have reached significance. Considering there was no change in preference for the control groups, this indicates that the auditory stimuli (music and white noise) used in these

experiments are not reinforcing to rats on there own, but potentiate the rewarding properties of MDMA.

MDMA is known to produce a CPP in rats, with optimal doses between 2.5 – 10 mg/kg (Bilsky et al., 1991; Herzig et al., 2005; Marona-Lewicka et al., 1996). Other non-drug factors, such as housing conditions and auditory stimuli, can impact MDMA's ability to drive place preference (Feduccia and Duvauchelle, 2008; Meyer et al., 2002). Although the underlying mechanisms by which MDMA induces place preference is not fully understood, a few studies have addressed this by looking at how different antagonists influence CPP. One study reported the effectiveness of a DA release inhibitor, which acts without occupying DA receptors, in blocking MDMA and cocaine CPP (Bilsky et al., 1998). Bilsky *et al*. demonstrated that MDL72222, a 5-HT_3 receptor antagonist, was effective in blocking MDMA CPP and theorized this effect is due to an inhibition of DA release via 5-HT_3 receptor antagonism (Bilsky and Reid, 1991). Additionally, the endocannabinoid and opioidergic systems play a role in mediating MDMA CPP, because pre-treatment of a CB_1 cannabinoid antagonist or naltrexone, an opioid antagonist, blocked place preference (Braida et al., 2005). Again, DA is indicated as the key player because cannabinoids and opioids regulate DA synthesis, release, and turnover (Gardner and Vorel, 1998). Taken together, MDMA's ability to establish a CPP is achieved by actions on multiple neural systems.

MDMA exhibits complex interactions on many substrates and circuits in the brain, including the DA and 5-HT systems. In the NAcc, MDMA elicits an increase in DA and to a greater extent 5-HT (Crespi et al., 1997; Johnson et al., 1986; Schmidt et al., 1987). Several studies have shown 5-HT neurotransmission to facilitate DA release via

activation of several different 5-HT receptor subtypes (Benloucif and Galloway, 1991; Bonhomme et al., 1995; Cameron and Williams, 1994). In our experiment, MDMA self-administered in conjunction with music produced the greatest increase in locomotor activity and extracellular NAcc DA and 5-HT. This study is the first to demonstrate that MDMA treatment with music can enhance neurochemical levels *in vivo* and has synergistic effects on the mesolimbic dopaminergic system.

In animals, MDMA can disrupt normal thermoregulatory mechanisms, causing hypothermia or hyperthermia, depending on experience, ambient temperature, and dose (Dafters, 1995; Malberg and Seiden, 1998). The degree of hyperthermia has been correlated with subsequent neurotoxicity (Malberg and Seiden, 1998). Both serotonin and dopamine are responsible for the hyperthermic response following MDMA administration, with D1 and 5-HT_{2A} receptors being the primary mediators (Shioda et al., 2008). Consistent with previous findings, our results showed high ambient temperatures increase the MDMA-induced hyperthermic response in rats. However, ambient temperature did not affect MDMA or saline self-administration rates or locomotor activity counts. An interesting finding from our experiments was that MDMA-stimulated NAcc 5-HT release, but not DA, was enhanced by self-administering the drug in a heated environment.

The studies presented in this dissertation demonstrate that environmental factors, namely auditory and thermal stimuli, can impact behavioral and neurochemical responses to low doses of MDMA. By taking this drug in noisy, hot conditions, human recreational users may experience heighten acute drug responses. However, most of the adverse side effects of MDMA are directly related to the drug-induced hyperthermia, which is

exacerbated in hot and crowded environments. Also, a gathering body of evidence indicates that music activates the same dopaminergic pathways as drugs of abuse and may lead to a state of over-arousal when accompanied with strong psychoactive drugs, such as MDMA. Further research is needed to understand how environmental stimuli present in the club and rave scenes impact the acute and long-term consequences of MDMA use.

Animal models are extremely useful in studying many avenues of neuroscience and for the development of treatments for human diseases and disorders. However, there are a limited number of animal behavioral tests available, especially for drug addiction research. The last part of this dissertation tested a novel method and approach for use in studying drug reinforcement in animals. Findings implicate that this approach can be used as a model for studying human drug consumption and reward and could also be used to test the effectiveness of behavioral and pharmacological therapies for addiction.

REFERENCES

Abikoff, H., Courtney, M.E., Szeibel, P.J., Koplewicz, H.S., 1996. The effects of auditory stimulation on the arithmetic performance of children with ADHD and nondisabled children. J Learn Disabil 29, 238-246.

Adams, J.U., Careri, J.M., Efferen, T.R., Rotrosen, J., 2001. Differential effects of dopamine antagonists on locomotor activity, conditioned activity and conditioned place preference induced by cocaine in rats. Behav Pharmacol 12, 603-611.

Adinoff, B., 2004. Neurobiologic processes in drug reward and addiction. Harv Rev Psychiatry 12, 305-320.

Angelucci, F., Fiore, M., Ricci, E., Padua, L., Sabino, A., Tonali, P.A., 2007a. Investigating the neurobiology of music: brain-derived neurotrophic factor modulation in the hippocampus of young adult mice. Behav Pharmacol 18, 491-496.

Angelucci, F., Ricci, E., Padua, L., Sabino, A., Tonali, P.A., 2007b. Music exposure differentially alters the levels of brain-derived neurotrophic factor and nerve growth factor in the mouse hypothalamus. Neurosci Lett 429, 152-155.

Arnold, J.M., Roberts, D.C., 1997. A critique of fixed and progressive ratio schedules used to examine the neural substrates of drug reinforcement. Pharmacol Biochem Behav 57, 441-447.

Avers, L., Mathur, A., Kamat, D., 2007. Music therapy in pediatrics. Clin Pediatr (Phila) 46, 575-579.

Badiani, A., Robinson, T.E., 2004. Drug-induced neurobehavioral plasticity: the role of environmental context. Behav Pharmacol 15, 327-339.

Bankson, M.G., Cunningham, K.A., 2001. 3,4-Methylenedioxymethamphetamine (MDMA) as a unique model of serotonin receptor function and serotonin-dopamine interactions. J Pharmacol Exp Ther 297, 846-852.

Bankson, M.G., Cunningham, K.A., 2002. Pharmacological studies of the acute effects of (+)-3,4-methylenedioxymethamphetamine on locomotor activity: role of 5-HT(1B/1D) and 5-HT(2) receptors. Neuropsychopharmacology 26, 40-52.

Bardo, M.T., 1998. Neuropharmacological mechanisms of drug reward: beyond dopamine in the nucleus accumbens. Crit Rev Neurobiol 12, 37-67.

Bardo, M.T., Bevins, R.A., 2000. Conditioned place preference: what does it add to our preclinical understanding of drug reward? Psychopharmacology (Berl) 153, 31-43.

Bardo, M.T., Rowlett, J.K., Harris, M.J., 1995. Conditioned place preference using opiate and stimulant drugs: a meta-analysis. Neurosci Biobehav Rev 19, 39-51.

Bardo, M.T., Valone, J.M., Bevins, R.A., 1999. Locomotion and conditioned place preference produced by acute intravenous amphetamine: role of dopamine receptors and individual differences in amphetamine self-administration. Psychopharmacology (Berl) 143, 39-46.

Battaglia, G., Yeh, S.Y., De Souza, E.B., 1988. MDMA-induced neurotoxicity: parameters of degeneration and recovery of brain serotonin neurons. Pharmacol Biochem Behav 29, 269-274.

Battaglia, G., Yeh, S.Y., O'Hearn, E., Molliver, M.E., Kuhar, M.J., De Souza, E.B., 1987. 3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: quantification of neurodegeneration by measurement of [3H]paroxetine-labeled serotonin uptake sites. J Pharmacol Exp Ther 242, 911-916.

Baumann, M.H., Clark, R.D., Rothman, R.B., 2008. Locomotor stimulation produced by 3,4-methylenedioxymethamphetamine (MDMA) is correlated with dialysate levels of serotonin and dopamine in rat brain. Pharmacol Biochem Behav.

Baumann, M.H., Wang, X., Rothman, R.B., 2006. 3,4-Methylenedioxymethamphetamine (MDMA) neurotoxicity in rats: a reappraisal of past and present findings. Psychopharmacology (Berl).

Bedi, G., Redman, J., 2006. Recreational ecstasy use: acute effects potentiated by ambient conditions? Neuropsychobiology 53, 113; author reply 114.

Benamar, K., Geller, E.B., Adler, M.W., 2008. A new brain area affected by 3,4-methylenedioxymethamphetamine: A microdialysis-biotelemetry study. Eur J Pharmacol 596, 84-88.

Benloucif, S., Galloway, M.P., 1991. Facilitation of dopamine release in vivo by serotonin agonists: studies with microdialysis. Eur J Pharmacol 200, 1-8.

Bernschneider-Reif, S., Oxler, F., Freudenmann, R.W., 2006. The origin of MDMA ("ecstasy")--separating the facts from the myth. Pharmazie 61, 966-972.

Bevins, R.A., Besheer, J., Palmatier, M.I., Jensen, H.C., Pickett, K.S., Eurek, S., 2002. Novel-object place conditioning: behavioral and dopaminergic processes in expression of novelty reward. Behav Brain Res 129, 41-50.

Bianchin, M., Mello e Souza, T., Medina, J.H., Izquierdo, I., 1999. The amygdala is involved in the modulation of long-term memory, but not in working or short-term memory. Neurobiol Learn Mem 71, 127-131.

Bilsky, E.J., Hubbell, C.L., Delconte, J.D., Reid, L.D., 1991. MDMA produces a conditioned place preference and elicits ejaculation in male rats: a modulatory role for the endogenous opioids. Pharmacol Biochem Behav 40, 443-447.

Bilsky, E.J., Hui, Y.Z., Hubbell, C.L., Reid, L.D., 1990. Methylenedioxymethamphetamine's capacity to establish place preferences and modify intake of an alcoholic beverage. Pharmacol Biochem Behav 37, 633-638.

Bilsky, E.J., Montegut, M.J., Nichols, M.L., Reid, L.D., 1998. CGS 10746B, a novel dopamine release inhibitor, blocks the establishment of cocaine and MDMA conditioned place preferences. Pharmacol Biochem Behav 59, 215-220.

Bilsky, E.J., Reid, L.D., 1991. MDL72222, a serotonin 5-HT3 receptor antagonist, blocks MDMA's ability to establish a conditioned place preference. Pharmacol Biochem Behav 39, 509-512.

Bjijou, Y., Stinus, L., Le Moal, M., Cador, M., 1996. Evidence for selective involvement of dopamine D1 receptors of the ventral tegmental area in the behavioral sensitization induced by intra-ventral tegmental area injections of D-amphetamine. J Pharmacol Exp Ther 277, 1177-1187.

Blackwell, h.r.S., H., 1943. Octave generalization, pitch discrimination, and loudness thresholds in the white rat. Journal of Experimental Psychology 33, 407-419.

Blood, A.J., Zatorre, R.J., 2001. Intensely pleasurable responses to music correlate with activity in brain regions implicated in reward and emotion. Proc Natl Acad Sci U S A 98, 11818-11823.

Bolla, K.I., McCann, U.D., Ricaurte, G.A., 1998. Memory impairment in abstinent MDMA ("Ecstasy") users. Neurology 51, 1532-1537.

Bonhomme, N., De Deurwaerdere, P., Le Moal, M., Spampinato, U., 1995. Evidence for 5-HT4 receptor subtype involvement in the enhancement of striatal dopamine release induced by serotonin: a microdialysis study in the halothane-anesthetized rat. Neuropharmacology 34, 269-279.

Bouso, J.C., Doblin, R., Farre, M., Alcazar, M.A., Gomez-Jarabo, G., 2008. MDMA-assisted psychotherapy using low doses in a small sample of women with chronic posttraumatic stress disorder. J Psychoactive Drugs 40, 225-236.

Bozarth, M.A., Gerber, G.J., Wise, R.A., 1980. Intracranial self-stimulation as a technique to study the reward properties of drugs of abuse. Pharmacol Biochem Behav 13 Suppl 1, 245-247.

Braida, D., Iosue, S., Pegorini, S., Sala, M., 2005. 3,4 Methylenedioxymethamphetamine-induced conditioned place preference (CPP) is mediated by endocannabinoid system. Pharmacol Res 51, 177-182.

Britton, K.T., Segal, D.S., Kuczenski, R., Hauger, R., 1992. Dissociation between in vivo hippocampal norepinephrine response and behavioral/neuroendocrine responses to noise stress in rats. Brain Res 574, 125-130.

Broening, H.W., Bowyer, J.F., Slikker, W., Jr., 1995. Age-dependent sensitivity of rats to the long-term effects of the serotonergic neurotoxicant (+/-)-3,4-methylenedioxymethamphetamine (MDMA) correlates with the magnitude of the MDMA-induced thermal response. J Pharmacol Exp Ther 275, 325-333.

Cadet, J.L., Thiriet, N., Jayanthi, S., 2001. Involvement of free radicals in MDMA-induced neurotoxicity in mice. Ann Med Interne (Paris) 152 Suppl 3, IS57-59.

Cadoni, C., Solinas, M., Pisanu, A., Zernig, G., Acquas, E., Di Chiara, G., 2005. Effect of 3,4-methylendioxymethamphetamine (MDMA, "ecstasy") on dopamine transmission in the nucleus accumbens shell and core. Brain Res 1055, 143-148.

Cagiano, R., Bera, I., Sabatini, R., Flace, P., Vermesan, D., Vermesan, H., Dragulescu, S.I., Bottalico, L., Santacroce, L., 2008. Effects on rat sexual behaviour of acute MDMA (ecstasy) alone or in combination with loud music. Eur Rev Med Pharmacol Sci 12, 285-292.

Cai, R., Guo, F., Zhang, J., Xu, J., Cui, Y., Sun, X., 2009. Environmental enrichment improves behavioral performance and auditory spatial representation of primary auditory cortical neurons in rat. Neurobiol Learn Mem 91, 366-376.

Callaway, C.W., Rempel, N., Peng, R.Y., Geyer, M.A., 1992. Serotonin 5-HT1-like receptors mediate hyperactivity in rats induced by 3,4-methylenedioxymethamphetamine. Neuropsychopharmacology 7, 113-127.

Callaway, C.W., Wing, L.L., Geyer, M.A., 1990. Serotonin release contributes to the locomotor stimulant effects of 3,4-methylenedioxymethamphetamine in rats. J Pharmacol Exp Ther 254, 456-464.

Cameron, D.L., Williams, J.T., 1994. Cocaine inhibits GABA release in the VTA through endogenous 5-HT. J Neurosci 14, 6763-6767.

Chen, Y.F., 2002. The Influence of Noise Stress-Induced pain-threshold increase on central monoaminergic neurons. Mid Taiwan J Med 7, 135-145.

Childress, A.R., Hole, A.V., Ehrman, R.N., Robbins, S.J., McLellan, A.T., O'Brien, C.P., 1993. Cue reactivity and cue reactivity interventions in drug dependence. NIDA Res Monogr 137, 73-95.

Cockerton, T., Moore, S., Norman, D., 1997. Cognitive test performance and background music. Percept Mot Skills 85, 1435-1438.

Colado, M.I., O'Shea, E., Esteban, B., Granados, R., Green, A.R., 1999. In vivo evidence against clomethiazole being neuroprotective against MDMA ('ecstasy')-induced degeneration of rat brain 5-HT nerve terminals by a free radical scavenging mechanism. Neuropharmacology 38, 307-314.

Colado, M.I., O'Shea, E., Granados, R., Murray, T.K., Green, A.R., 1997. In vivo evidence for free radical involvement in the degeneration of rat brain 5-HT following administration of MDMA ('ecstasy') and p-chloroamphetamine but not the degeneration following fenfluramine. Br J Pharmacol 121, 889-900.

Cole, J.C., Sumnall, H.R., 2003. The pre-clinical behavioural pharmacology of 3,4-methylenedioxymethamphetamine (MDMA). Neurosci Biobehav Rev 27, 199-217.

Collins, R.J., Weeks, J.R., Cooper, M.M., Good, P.I., Russell, R.R., 1984. Prediction of abuse liability of drugs using IV self-administration by rats. Psychopharmacology (Berl) 82, 6-13.

Commins, D.L., Vosmer, G., Virus, R.M., Woolverton, W.L., Schuster, C.R., Seiden, L.S., 1987. Biochemical and histological evidence that methylenedioxymethylamphetamine (MDMA) is toxic to neurons in the rat brain. J Pharmacol Exp Ther 241, 338-345.

Connor, T.J., 2004. Methylenedioxymethamphetamine (MDMA, 'Ecstasy'): a stressor on the immune system. Immunology 111, 357-367.

Connor, T.J., McNamara, M.G., Finn, D., Currid, A., O'Malley, M., Redmond, A.M., Kelly, J.P., Leonard, B.E., 1998. Acute 3,4-methylenedioxymethamphetamine(MDMA) administration produces a rapid and sustained suppression of immune function in the rat. Immunopharmacology 38, 253-260.

Cornish, J.L., Shahnawaz, Z., Thompson, M.R., Wong, S., Morley, K.C., Hunt, G.E., McGregor, I.S., 2003. Heat increases 3,4-methylenedioxymethamphetamine self-administration and social effects in rats. Eur J Pharmacol 482, 339-341.

Cowan, R.L., Lyoo, I.K., Sung, S.M., Ahn, K.H., Kim, M.J., Hwang, J., Haga, E., Vimal, R.L., Lukas, S.E., Renshaw, P.F., 2003. Reduced cortical gray matter density in human MDMA (Ecstasy) users: a voxel-based morphometry study. Drug Alcohol Depend 72, 225-235.

Crespi, D., Mennini, T., Gobbi, M., 1997. Carrier-dependent and Ca(2+)-dependent 5-HT and dopamine release induced by (+)-amphetamine, 3,4-methylendioxymethamphetamine, p-chloroamphetamine and (+)-fenfluramine. Br J Pharmacol 121, 1735-1743.

Crowe, S.F., Ng, K.T., Gibbs, M.E., 1990. Memory consolidation of weak training experiences by hormonal treatments. Pharmacol Biochem Behav 37, 729-734.

Cunningham, C.L., Dickinson, S.D., Grahame, N.J., Okorn, D.M., McMullin, C.S., 1999. Genetic differences in cocaine-induced conditioned place preference in mice depend on conditioning trial duration. Psychopharmacology (Berl) 146, 73-80.

Curran, H.V., Rees, H., Hoare, T., Hoshi, R., Bond, A., 2004. Empathy and aggression: two faces of ecstasy? A study of interpretative cognitive bias and mood change in ecstasy users. Psychopharmacology (Berl) 173, 425-433.

Dafters, R.I., 1994. Effect of ambient temperature on hyperthermia and hyperkinesis induced by 3,4-methylenedioxymethamphetamine (MDMA or "ecstasy") in rats. Psychopharmacology (Berl) 114, 505-508.

Dafters, R.I., 1995. Hyperthermia following MDMA administration in rats: effects of ambient temperature, water consumption, and chronic dosing. Physiol Behav 58, 877-882.

Daniela, E., Brennan, K., Gittings, D., Hely, L., Schenk, S., 2004. Effect of SCH 23390 on (+/-)-3,4-methylenedioxymethamphetamine hyperactivity and self-administration in rats. Pharmacol Biochem Behav 77, 745-750.

Daza-Losada, M., Ribeiro Do Couto, B., Manzanedo, C., Aguilar, M.A., Rodriguez-Arias, M., Minarro, J., 2007. Rewarding effects and reinstatement of MDMA-induced CPP in adolescent mice. Neuropsychopharmacology 32, 1750-1759.

De La Garza, R., 2nd, Fabrizio, K.R., Gupta, A., 2006. Relevance of rodent models of intravenous MDMA self-administration to human MDMA consumption patterns. Psychopharmacology (Berl), 1-10.

de la Torre, R., Farre, M., Roset, P.N., Lopez, C.H., Mas, M., Ortuno, J., Menoyo, E., Pizarro, N., Segura, J., Cami, J., 2000. Pharmacology of MDMA in humans. Ann N Y Acad Sci 914, 225-237.

Dela Cruz, A.M., Herin, D.V., Grady, J.J., Cunningham, K.A., 2009. Novel approach to data analysis in cocaine-conditioned place preference. Behav Pharmacol.

Dellu, F., Piazza, P.V., Mayo, W., Le Moal, M., Simon, H., 1996. Novelty-seeking in rats--biobehavioral characteristics and possible relationship with the sensation-seeking trait in man. Neuropsychobiology 34, 136-145.

Deroche, V., Le Moal, M., Piazza, P.V., 1999. Cocaine self-administration increases the incentive motivational properties of the drug in rats. Eur J Neurosci 11, 2731-2736.

Di Chiara, G., Bassareo, V., Fenu, S., De Luca, M.A., Spina, L., Cadoni, C., Acquas, E., Carboni, E., Valentini, V., Lecca, D., 2004. Dopamine and drug addiction: the nucleus accumbens shell connection. Neuropharmacology 47 Suppl 1, 227-241.

Di Ciano, P., Blaha, C.D., Phillips, A.G., 1998. Conditioned changes in dopamine oxidation currents in the nucleus accumbens of rats by stimuli paired with self-administration or yoked-administration of d-amphetamine. Eur J Neurosci 10, 1121-1127.

Dickerson, S.M., Walker, D.M., Reveron, M.E., Duvauchelle, C.L., Gore, A.C., 2008. The recreational drug ecstasy disrupts the hypothalamic-pituitary-gonadal reproductive axis in adult male rats. Neuroendocrinology 88, 95-102.

Dimicco, J.A., Zaretsky, D.V., 2007. The dorsomedial hypothalamus: a new player in thermoregulation. Am J Physiol Regul Integr Comp Physiol 292, R47-63.

Duvauchelle, C.L., Ikegami, A., Castaneda, E., 2000. Conditioned increases in behavioral activity and accumbens dopamine levels produced by intravenous cocaine. Behav Neurosci 114, 1156-1166.

Emanuele, E., Arra, M., Pesenti, S., 2006. Vasopressin and oxytocin as neurohormonal mediators of MDMA (ecstasy) sociosexual behavioural effects. Med Hypotheses 67, 1250-1251.

Ettenberg, A., Raven, M.A., Danluck, D.A., Necessary, B.D., 1999. Evidence for opponent-process actions of intravenous cocaine. Pharmacol Biochem Behav 64, 507-512.

Fallon, J.K., Shah, D., Kicman, A.T., Hutt, A.J., Henry, J.A., Cowan, D.A., Forsling, M., 2002. Action of MDMA (ecstasy) and its metabolites on arginine vasopressin release. Ann N Y Acad Sci 965, 399-409.

Fang, G.Z., Zhang, C.P., Wu, D., Xia, Y., Lai, Y.X., Yao, D.Z., 2009. Increased Arousal Levels and Decreased Sleep by Brain Music in Rats

. JOURNAL OF ELECTRONIC SCIENCE AND TECHNOLOGY OF CHINA 7, 40-46.

Fantegrossi, W.E., Woolverton, W.L., Kilbourn, M., Sherman, P., Yuan, J., Hatzidimitriou, G., Ricaurte, G.A., Woods, J.H., Winger, G., 2004. Behavioral and neurochemical consequences of long-term intravenous self-administration of MDMA and its enantiomers by rhesus monkeys. Neuropsychopharmacology 29, 1270-1281.

Feduccia, A.A., Duvauchelle, C.L., 2008. Auditory stimuli enhances MDMA-conditioned reward and MDMA-induced nucleus accumbens dopamine, serotonin and locomotor responses. Brain Res Bull.

Fischer, C., Hatzidimitriou, G., Wlos, J., Katz, J., Ricaurte, G., 1995. Reorganization of ascending 5-HT axon projections in animals previously exposed to the recreational drug (+/-)3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). J Neurosci 15, 5476-5485.

Fischman, M.W., Schuster, C.R., 1978. Drug seeking: a behavioral analysis in animals and humans. NIDA Res Monogr, 4-23.

Fitch, W.T., 2005. The evolution of music in comparative perspective. Ann N Y Acad Sci 1060, 29-49.

Fitch, W.T., 2006. The biology and evolution of music: a comparative perspective. Cognition 100, 173-215.

Forsling, M.L., Fallon, J.K., Shah, D., Tilbrook, G.S., Cowan, D.A., Kicman, A.T., Hutt, A.J., 2002. The effect of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') and its metabolites on neurohypophysial hormone release from the isolated rat hypothalamus. Br J Pharmacol 135, 649-656.

Gardner, E.L., 2000. What we have learned about addiction from animal models of drug self-administration. Am J Addict 9, 285-313.

Gardner, E.L., Vorel, S.R., 1998. Cannabinoid transmission and reward-related events. Neurobiol Dis 5, 502-533.

Gesi, M., Ferrucci, M., Giusiani, M., Lenzi, P., Lazzeri, G., Alessandri, M.G., Salvadorini, A., Fulceri, F., Pellegrini, A., Fornai, F., Paparelli, A., 2004. Loud noise enhances nigrostriatal dopamine toxicity induced by MDMA in mice. Microsc Res Tech 64, 297-303.

Gillman, P.K., 1998. Serotonin syndrome: history and risk. Fundam Clin Pharmacol 12, 482-491.

Gillman, P.K., 1999. The serotonin syndrome and its treatment. J Psychopharmacol 13, 100-109.

Glennon, R.A., Higgs, R., 1992. Investigation of MDMA-related agents in rats trained to discriminate MDMA from saline. Pharmacol Biochem Behav 43, 759-763.

Gold, C., 2007. Music therapy improves symptoms in adults hospitalised with schizophrenia. Evid Based Ment Health 10, 77.

Gold, L.H., Geyer, M.A., Koob, G.F., 1989. Psychostimulant properties of MDMA. NIDA Res Monogr 95, 345-346.

Gold, L.H., Koob, G.F., 1989. MDMA produces stimulant-like conditioned locomotor activity. Psychopharmacology (Berl) 99, 352-356.

Gough, B., Ali, S.F., Slikker, W., Jr., Holson, R.R., 1991. Acute effects of 3,4-methylenedioxymethamphetamine (MDMA) on monoamines in rat caudate. Pharmacol Biochem Behav 39, 619-623.

Green, A.R., Cross, A.J., Goodwin, G.M., 1995. Review of the pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA or "Ecstasy"). Psychopharmacology (Berl) 119, 247-260.

Green, A.R., Mechan, A.O., Elliott, J.M., O'Shea, E., Colado, M.I., 2003. The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). Pharmacol Rev 55, 463-508.

Green, A.R., O'Shea, E., Colado, M.I., 2004a. A review of the mechanisms involved in the acute MDMA (ecstasy)-induced hyperthermic response. Eur J Pharmacol 500, 3-13.

Green, A.R., Sanchez, V., O'Shea, E., Saadat, K.S., Elliott, J.M., Colado, M.I., 2004b. Effect of ambient temperature and a prior neurotoxic dose of 3,4-methylenedioxymethamphetamine (MDMA) on the hyperthermic response of rats to a single or repeated ('binge' ingestion) low dose of MDMA. Psychopharmacology (Berl) 173, 264-269.

Gudelsky, G.A., Nash, J.F., 1996. Carrier-mediated release of serotonin by 3,4-methylenedioxymethamphetamine: implications for serotonin-dopamine interactions. J Neurochem 66, 243-249.

Gudelsky, G.A., Yamamoto, B.K., 2008. Actions of 3,4-methylenedioxymethamphetamine (MDMA) on cerebral dopaminergic, serotonergic and cholinergic neurons. Pharmacol Biochem Behav 90, 198-207.

Hall, A.P., Henry, J.A., 2006. Acute toxic effects of 'Ecstasy' (MDMA) and related compounds: overview of pathophysiology and clinical management. Br J Anaesth 96, 678-685.

Halliwell, B., Kaur, H., 1997. Hydroxylation of salicylate and phenylalanine as assays for hydroxyl radicals: a cautionary note visited for the third time. Free Radic Res 27, 239-244.

Halliwell, B., Kaur, H., Ingelman-Sundberg, M., 1991. Hydroxylation of salicylate as an assay for hydroxyl radicals: a cautionary note. Free Radic Biol Med 10, 439-441.

Hardman, H.F., Haavik, C.O., Seevers, M.H., 1973. Relationship of the structure of mescaline and seven analogs to toxicity and behavior in five species of laboratory animals. Toxicol Appl Pharmacol 25, 299-309.

Hargreaves, G.A., Hunt, G.E., Cornish, J.L., McGregor, I.S., 2007. High ambient temperature increases 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy")-induced Fos expression in a region-specific manner. Neuroscience 145, 764-774.

Hauser, M.D., McDermott, J., 2003. The evolution of the music faculty: a comparative perspective. Nat Neurosci 6, 663-668.

Henry, J.A., Fallon, J.K., Kicman, A.T., Hutt, A.J., Cowan, D.A., Forsling, M., 1998. Low-dose MDMA ("ecstasy") induces vasopressin secretion. Lancet 351, 1784.

Henry, J.A., Jeffreys, K.J., Dawling, S., 1992. Toxicity and deaths from 3,4-methylenedioxymethamphetamine ("ecstasy"). Lancet 340, 384-387.

Hermle, L., Spitzer, M., Borchardt, D., Kovar, K.A., Gouzoulis, E., 1993. Psychological effects of MDE in normal subjects. Are entactogens a new class of psychoactive agents? Neuropsychopharmacology 8, 171-176.

Herzig, V., Capuani, E.M., Kovar, K.A., Schmidt, W.J., 2005. Effects of MPEP on expression of food-, MDMA- or amphetamine-conditioned place preference in rats. Addict Biol 10, 243-249.

Hiramatsu, M., Nabeshima, T., Kameyama, T., Maeda, Y., Cho, A.K., 1989. The effect of optical isomers of 3,4-methylenedioxymethamphetamine (MDMA) on stereotyped behavior in rats. Pharmacol Biochem Behav 33, 343-347.

Hooks, M.S., Colvin, A.C., Juncos, J.L., Justice, J.B., Jr., 1992. Individual differences in basal and cocaine-stimulated extracellular dopamine in the nucleus accumbens using quantitative microdialysis. Brain Res 587, 306-312.

Hooks, M.S., Juncos, J.L., Justice, J.B., Jr., Meiergerd, S.M., Povlock, S.L., Schenk, J.O., Kalivas, P.W., 1994. Individual locomotor response to novelty predicts selective alterations in D1 and D2 receptors and mRNAs. J Neurosci 14, 6144-6152.

House, R.V., Thomas, P.T., Bhargava, H.N., 1995. Selective modulation of immune function resulting from in vitro exposure to methylenedioxymethamphetamine (Ecstasy). Toxicology 96, 59-69.

Huether, G., Zhou, D., Ruther, E., 1997. Causes and consequences of the loss of serotonergic presynapses elicited by the consumption of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") and its congeners. J Neural Transm 104, 771-794.

Hulse, S.H., Humpal, J., Cynx, J., 1984. Processing of rhythmic sound structures by birds. Ann N Y Acad Sci 423, 407-419.

Iannone, M., Bulotta, S., Paolino, D., Zito, M.C., Gratteri, S., Costanzo, F.S., Rotiroti, D., 2006. Electrocortical effects of MDMA are potentiated by acoustic stimulation in rats. BMC Neurosci 7, 13.

Ikegami, A., Olsen, C.M., D'Souza, M.S., Duvauchelle, C.L., 2007. Experience-dependent effects of cocaine self-administration/conditioning on prefrontal and accumbens dopamine responses. Behav Neurosci 121, 389-400.

Indlekofer, F., Piechatzek, M., Daamen, M., Glasmacher, C., Lieb, R., Pfister, H., Tucha, O., Lange, K., Wittchen, H., Schutz, C., 2009. Reduced memory and attention performance in a population-based sample of young adults with a moderate lifetime use of cannabis, ecstasy and alcohol. J Psychopharmacol 23, 495-509.

Johnson, M.P., Hoffman, A.J., Nichols, D.E., 1986. Effects of the enantiomers of MDA, MDMA and related analogues on [3H]serotonin and [3H]dopamine release from superfused rat brain slices. Eur J Pharmacol 132, 269-276.

Kabbaj, M., 2004. Neurobiological bases of individual differences in emotional and stress responsiveness: high responders-low responders model. Arch Neurol 61, 1009-1012.

Kabbaj, M., 2006. Individual differences in vulnerability to drug abuse: the high responders/low responders model. CNS Neurol Disord Drug Targets 5, 513-520.

Kabbaj, M., Devine, D.P., Savage, V.R., Akil, H., 2000. Neurobiological correlates of individual differences in novelty-seeking behavior in the rat: differential expression of stress-related molecules. J Neurosci 20, 6983-6988.

Kabbaj, M., Morley-Fletcher, S., Le Moal, M., Maccari, S., 2007. Individual differences in the effects of chronic prazosin hydrochloride treatment on hippocampal mineralocorticoid and glucocorticoid receptors. Eur J Neurosci 25, 3312-3318.

Kalant, H., 2001. The pharmacology and toxicology of "ecstasy" (MDMA) and related drugs. Cmaj 165, 917-928.

Kalivas, P.W., Duffy, P., 1990. Effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. Synapse 5, 48-58.

Kalivas, P.W., Duffy, P., White, S.R., 1998. MDMA elicits behavioral and neurochemical sensitization in rats. Neuropsychopharmacology 18, 469-479.

Kankaanpaa, A., Meririnne, E., Lillsunde, P., Seppala, T., 1998. The acute effects of amphetamine derivatives on extracellular serotonin and dopamine levels in rat nucleus accumbens. Pharmacol Biochem Behav 59, 1003-1009.

Kanosue, K., Yanase-Fujiwara, M., Hosono, T., 1994a. Hypothalamic network for thermoregulatory vasomotor control. Am J Physiol 267, R283-288.

Kanosue, K., Zhang, Y.H., Yanase-Fujiwara, M., Hosono, T., 1994b. Hypothalamic network for thermoregulatory shivering. Am J Physiol 267, R275-282.

Kehne, J.H., Ketteler, H.J., McCloskey, T.C., Sullivan, C.K., Dudley, M.W., Schmidt, C.J., 1996. Effects of the selective 5-HT2A receptor antagonist MDL 100,907 on MDMA-induced locomotor stimulation in rats. Neuropsychopharmacology 15, 116-124.

Koch, S., Galloway, M.P., 1997. MDMA induced dopamine release in vivo: role of endogenous serotonin. J Neural Transm 104, 135-146.

Koob, G.F., 1995. Animal models of drug addiction. . In: Bloom, K.D. (Ed.), Psychopharmacology: the fourth generation of progress. Raven Press, New York, pp. 759-772.

Kurling, S., Kankaanpaa, A., Seppala, T., 2008. Sub-chronic nandrolone treatment modifies neurochemical and behavioral effects of amphetamine and 3,4-methylenedioxymethamphetamine (MDMA) in rats. Behav Brain Res 189, 191-201.

Kutscher, C.L., Yamamoto, B.K., 1979. A frequency analysis of behavior components of the serotonin syndrome produced by p-chloroamphetamine. Pharmacol Biochem Behav 11, 611-616.

Lile, J.A., Ross, J.T., Nader, M.A., 2005. A comparison of the reinforcing efficacy of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") with cocaine in rhesus monkeys. Drug Alcohol Depend 78, 135-140.

Line, S., Markowitz, H., Morgan, K.N., & Strong, S., 1991. Through the looking glass: Issues of psychological wellbeing in captive nonhuman primates. American Psychological Association, Washington, D.C.

Liu, Y., Roberts, D.C., Morgan, D., 2005. Sensitization of the reinforcing effects of self-administered cocaine in rats: effects of dose and intravenous injection speed. Eur J Neurosci 22, 195-200.

Malberg, J.E., Seiden, L.S., 1998. Small changes in ambient temperature cause large changes in 3,4-methylenedioxymethamphetamine (MDMA)-induced serotonin neurotoxicity and core body temperature in the rat. J Neurosci 18, 5086-5094.

Marona-Lewicka, D., Rhee, G.S., Sprague, J.E., Nichols, D.E., 1996. Reinforcing effects of certain serotonin-releasing amphetamine derivatives. Pharmacol Biochem Behav 53, 99-105.

Mayer, L.A., Parker, L.A., 1993. Rewarding and aversive properties of IP and SC cocaine: assessment by place and taste conditioning. Psychopharmacology (Berl) 112, 189-194.

McAllen, R.M., May, C.N., 1994. Effects of preoptic warming on subretrofacial and cutaneous vasoconstrictor neurons in anaesthetized cats. J Physiol 481 (Pt 3), 719-730.

McCreary, A.C., Bankson, M.G., Cunningham, K.A., 1999. Pharmacological studies of the acute and chronic effects of (+)-3, 4-methylenedioxymethamphetamine on locomotor activity: role of 5-hydroxytryptamine(1A) and 5-hydroxytryptamine(1B/1D) receptors. J Pharmacol Exp Ther 290, 965-973.

McElrath, K., McEvoy, K., 2002. Negative experiences on Ecstasy: the role of drug, set and setting. J Psychoactive Drugs 34, 199-208.

Mechan, A.O., Esteban, B., O'Shea, E., Elliott, J.M., Colado, M.I., Green, A.R., 2002. The pharmacology of the acute hyperthermic response that follows administration of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') to rats. Br J Pharmacol 135, 170-180.

Meisch, R.A., 2001. Oral drug self-administration: an overview of laboratory animal studies. Alcohol 24, 117-128.

Menon, V., Levitin, D.J., 2005. The rewards of music listening: response and physiological connectivity of the mesolimbic system. Neuroimage 28, 175-184.

Meyer, A., Mayerhofer, A., Kovar, K.A., Schmidt, W.J., 2002. Rewarding effects of the optical isomers of 3,4-methylenedioxy-methylamphetamine ('Ecstasy') and 3,4-methylenedioxy-ethylamphetamine ('Eve') measured by conditioned place preference in rats. Neurosci Lett 330, 280-284.

Miguens, M., Crespo, J.A., Del Olmo, N., Higuera-Matas, A., Montoya, G.L., Garcia-Lecumberri, C., Ambrosio, E., 2008. Differential cocaine-induced modulation of glutamate and dopamine transporters after contingent and non-contingent administration. Neuropharmacology 55, 771-779.

Mills, E.M., Banks, M.L., Sprague, J.E., Finkel, T., 2003. Pharmacology: uncoupling the agony from ecstasy. Nature 426, 403-404.

Moolten, M., Kornetsky, C., 1990. Oral self-administration of ethanol and not experimenter-administered ethanol facilitates rewarding electrical brain stimulation. Alcohol 7, 221-225.

Morgan, M.J., 2000. Ecstasy (MDMA): a review of its possible persistent psychological effects. Psychopharmacology (Berl) 152, 230-248.

Morrison, S.F., 2001a. Differential regulation of brown adipose and splanchnic sympathetic outflows in rat: roles of raphe and rostral ventrolateral medulla neurons. Clin Exp Pharmacol Physiol 28, 138-143.

Morrison, S.F., 2001b. Differential regulation of sympathetic outflows to vasoconstrictor and thermoregulatory effectors. Ann N Y Acad Sci 940, 286-298.

Morton, A.J., Hickey, M.A., Dean, L.C., 2001. Methamphetamine toxicity in mice is potentiated by exposure to loud music. Neuroreport 12, 3277-3281.

Nagashima, K., Nakai, S., Tanaka, M., Kanosue, K., 2000. Neuronal circuitries involved in thermoregulation. Auton Neurosci 85, 18-25.

Nash, J.F., Jr., Meltzer, H.Y., Gudelsky, G.A., 1988. Elevation of serum prolactin and corticosterone concentrations in the rat after the administration of 3,4-methylenedioxymethamphetamine. J Pharmacol Exp Ther 245, 873-879.

Nestler, E.J., 2004. Molecular mechanisms of drug addiction. Neuropharmacology 47 Suppl 1, 24-32.

Nomikos, G.G., Spyraki, C., 1988. Cocaine-induced place conditioning: importance of route of administration and other procedural variables. Psychopharmacology (Berl) 94, 119-125.

O'Brien, C., Childress, A.R., Ehrman, R., Robbins, S., McLellan, A.T., 1992. Conditioning mechanisms in drug dependence. Clin Neuropharmacol 15 Suppl 1 Pt A, 66A-67A.

O'Dell, L.E., Khroyan, T.V., Neisewander, J.L., 1996. Dose-dependent characterization of the rewarding and stimulant properties of cocaine following intraperitoneal and intravenous administration in rats. Psychopharmacology (Berl) 123, 144-153.

O'Hearn, E., Battaglia, G., De Souza, E.B., Kuhar, M.J., Molliver, M.E., 1988. Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity. J Neurosci 8, 2788-2803.

O'Shea, E., Escobedo, I., Orio, L., Sanchez, V., Navarro, M., Green, A.R., Colado, M.I., 2005. Elevation of ambient room temperature has differential effects on MDMA-induced 5-HT and dopamine release in striatum and nucleus accumbens of rats. Neuropsychopharmacology 30, 1312-1323.

O'Shea, E., Granados, R., Esteban, B., Colado, M.I., Green, A.R., 1998. The relationship between the degree of neurodegeneration of rat brain 5-HT nerve terminals and the dose and frequency of administration of MDMA ('ecstasy'). Neuropharmacology 37, 919-926.

Oberlender, R., Nichols, D.E., 1988. Drug discrimination studies with MDMA and amphetamine. Psychopharmacology (Berl) 95, 71-76.

Olds, M.E., Christenson, T., 1970. Effects of drive and training on extinction after self-stimulation and food reward. Am J Physiol 219, 208-213.

Pacifici, R., Zuccaro, P., Hernandez Lopez, C., Pichini, S., Di Carlo, S., Farre, M., Roset, P.N., Ortuno, J., Segura, J., Torre, R.L., 2001. Acute effects of 3,4-methylenedioxymethamphetamine alone and in combination with ethanol on the immune system in humans. J Pharmacol Exp Ther 296, 207-215.

Page, S.C., Hulse, S.H., Cynx, J., 1989. Relative pitch perception in the European starling (Sturnus vulgaris): further evidence for an elusive phenomenon. J Exp Psychol Anim Behav Process 15, 137-146.

Palamarchouk, V., Smagin, G., Goeders, N.E., 2009. Self-administered and passive cocaine infusions produce different effects on corticosterone concentrations in the medial prefrontal cortex (MPC) of rats. Pharmacol Biochem Behav 94, 163-168.

Panksepp, J., Bernatzky, G., 2002. Emotional sounds and the brain: the neuro-affective foundations of musical appreciation. Behav Processes 60, 133-155.

Panlilio, L.V., Goldberg, S.R., 2007. Self-administration of drugs in animals and humans as a model and an investigative tool. Addiction 102, 1863-1870.

Panlilio, L.V., Yasar, S., Nemeth-Coslett, R., Katz, J.L., Henningfield, J.E., Solinas, M., Heishman, S.J., Schindler, C.W., Goldberg, S.R., 2005. Human cocaine-seeking behavior and its control by drug-associated stimuli in the laboratory. Neuropsychopharmacology 30, 433-443.

Parrott, A.C., 2002. Recreational Ecstasy/MDMA, the serotonin syndrome, and serotonergic neurotoxicity. Pharmacol Biochem Behav 71, 837-844.

Parrott, A.C., 2004. MDMA (3,4-Methylenedioxymethamphetamine) or ecstasy: the neuropsychobiological implications of taking it at dances and raves. Neuropsychobiology 50, 329-335.

Parrott, A.C., 2005. Chronic tolerance to recreational MDMA (3,4-methylenedioxymethamphetamine) or Ecstasy. J Psychopharmacol 19, 71-83.

Parrott, A.C., Buchanan, T., Scholey, A.B., Heffernan, T., Ling, J., Rodgers, J., 2002. Ecstasy/MDMA attributed problems reported by novice, moderate and heavy recreational users. Hum Psychopharmacol 17, 309-312.

Parrott, A.C., Lock, J., Conner, A.C., Kissling, C., Thome, J., 2008. Dance clubbing on MDMA and during abstinence from Ecstasy/MDMA: prospective neuroendocrine and psychobiological changes. Neuropsychobiology 57, 165-180.

Parrott, A.C., Rodgers, J., Buchanan, T., Ling, J., Heffernan, T., Scholey, A.B., 2006. Dancing hot on Ecstasy: physical activity and thermal comfort ratings are associated with

the memory and other psychobiological problems reported by recreational MDMA users. Hum Psychopharmacol 21, 285-298.

Paxinos, g., Watson, c., 1997. The rat brain: in stereotaxic coordinates (atlas & Computer Graphics Files), 3 ed. ed. Academic Press, Inc., San Diego.

Peretti, P.O.K., H., 1991. influence of five types of music on social behaviors of mice. Indian Journal of Behavior 15, 133-155.

Pettit, H.O., Justice, J.B., Jr., 1991. Effect of dose on cocaine self-administration behavior and dopamine levels in the nucleus accumbens. Brain Res 539, 94-102.

Philipkoski, K., 2004. DEA Accedes to Ecstasy Test. Wired.

Piazza, P.V., Deminiere, J.M., Le Moal, M., Simon, H., 1989. Factors that predict individual vulnerability to amphetamine self-administration. Science 245, 1511-1513.

Piazza, P.V., Deroche-Gamonent, V., Rouge-Pont, F., Le Moal, M., 2000. Vertical shifts in self-administration dose-response functions predict a drug-vulnerable phenotype predisposed to addiction. J Neurosci 20, 4226-4232.

Piazza, P.V., Le Moal, M., 1998. The role of stress in drug self-administration. Trends Pharmacol Sci 19, 67-74.

Piazza, P.V., Maccari, S., Deminiere, J.M., Le Moal, M., Mormede, P., Simon, H., 1991a. Corticosterone levels determine individual vulnerability to amphetamine self-administration. Proc Natl Acad Sci U S A 88, 2088-2092.

Piazza, P.V., Rouge-Pont, F., Deminiere, J.M., Kharoubi, M., Le Moal, M., Simon, H., 1991b. Dopaminergic activity is reduced in the prefrontal cortex and increased in the nucleus accumbens of rats predisposed to develop amphetamine self-administration. Brain Res 567, 169-174.

Piper, B.J., Fraiman, J.B., Meyer, J.S., 2005. Repeated MDMA ("Ecstasy") exposure in adolescent male rats alters temperature regulation, spontaneous motor activity, attention, and serotonin transporter binding. Dev Psychobiol 47, 145-157.

Prior, H., 2006. Effects of the acoustic environment on learning in rats. Physiol Behav 87, 162-165.

Quednow, B.B., Jessen, F., Kuhn, K.U., Maier, W., Daum, I., Wagner, M., 2006. Memory deficits in abstinent MDMA (ecstasy) users: neuropsychological evidence of frontal dysfunction. J Psychopharmacol 20, 373-384.

Quinton, M.S., Yamamoto, B.K., 2006. Causes and consequences of methamphetamine and MDMA toxicity. Aaps J 8, E337-347.

Raman, E.R., Vanhuyse, V.J., Roberts, M.F., 1987. Mathematical circulation model for the blood-flow-heat-loss relationship in the rat tail. Phys Med Biol 32, 859-875.

Randall, J.A., 1997. Species-specific footdrumming in kangaroo rats: Dipodomys ingens, D. deserti, D. spectabilis. Anim Behav 54, 1167-1175.

Randall, J.A., 2001. Evolution and function of drumming as communicaiton in mammals. Amer Zool, 1143-1156.

Randall, J.A., Lewis, E.R., 1997. Seismic communication between the burrows of kangaroo rats, Dipodomys spectabilis. J Comp Physiol A 181, 525-531.

Rathner, J.A., McAllen, R.M., 1999. Differential control of sympathetic drive to the rat tail artery and kidney by medullary premotor cell groups. Brain Res 834, 196-199.

Ratzenboeck, E., Saria, A., Kriechbaum, N., Zernig, G., 2001. Reinforcing effects of MDMA ("ecstasy") in drug-naive and cocaine-trained rats. Pharmacology 62, 138-144.

Remedios, R., Logothetis, N.K., Kayser, C., 2009. Monkey drumming reveals common networks for perceiving vocal and nonvocal communication sounds. Proc Natl Acad Sci U S A 106, 18010-18015.

Rempel, N.L., Callaway, C.W., Geyer, M.A., 1993. Serotonin1B receptor activation mimics behavioral effects of presynaptic serotonin release. Neuropsychopharmacology 8, 201-211.

Reneman, L., Endert, E., de Bruin, K., Lavalaye, J., Feenstra, M.G., de Wolff, F.A., Booij, J., 2002. The acute and chronic effects of MDMA ("ecstasy") on cortical 5-HT2A receptors in rat and human brain. Neuropsychopharmacology 26, 387-396.

Reveron, M.E., Maier, E.Y., Duvauchelle, C.L., 2006. Experience-dependent changes in temperature and behavioral activity induced by MDMA. Physiol Behav.

Ricaurte, G.A., Yuan, J., Hatzidimitriou, G., Cord, B.J., McCann, U.D., 2002. Severe dopaminergic neurotoxicity in primates after a common recreational dose regimen of MDMA ("ecstasy"). Science 297, 2260-2263.

Ricaurte, G.A., Yuan, J., Hatzidimitriou, G., Cord, B.J., McCann, U.D., 2003. Retraction. Science 301, 1479.

Richards, D.G., Wolz, J.P., Herman, L.M., 1984. Vocal mimicry of computer-generated sounds and vocal labeling of objects by a bottlenosed dolphin, Tursiops truncatus. J Comp Psychol 98, 10-28.

Rickard, N.S., 2009. Defining the rhythmicity of memory-enhancing acoustic stimuli in the young domestic chick (Gallus gallus). J Comp Psychol 123, 217-221.

Rickard, N.S., Toukhsati, S.R., Field, S.E., 2005. The effect of music on cognitive performance: insight from neurobiological and animal studies. Behav Cogn Neurosci Rev 4, 235-261.

Ritz, M.C., Kuhar, M.J., 1993. Psychostimulant drugs and a dopamine hypothesis regarding addiction: update on recent research. Biochem Soc Symp 59, 51-64.

Roberts, D.C., Morgan, D., Liu, Y., 2007. How to make a rat addicted to cocaine. Prog Neuropsychopharmacol Biol Psychiatry 31, 1614-1624.

Rocha, B.A., Fumagalli, F., Gainetdinov, R.R., Jones, S.R., Ator, R., Giros, B., Miller, G.W., Caron, M.G., 1998. Cocaine self-administration in dopamine-transporter knockout mice. Nat Neurosci 1, 132-137.

Rudnick, G., Wall, S.C., 1992. The molecular mechanism of "ecstasy" [3,4-methylenedioxy-methamphetamine (MDMA)]: serotonin transporters are targets for MDMA-induced serotonin release. Proc Natl Acad Sci U S A 89, 1817-1821.

Russo, S.J., Jenab, S., Fabian, S.J., Festa, E.D., Kemen, L.M., Quinones-Jenab, V., 2003. Sex differences in the conditioned rewarding effects of cocaine. Brain Res 970, 214-220.

Rusyniak, D.E., Ootsuka, Y., Blessing, W.W., 2008. When administered to rats in a cold environment, 3,4-methylenedioxymethamphetamine reduces brown adipose tissue thermogenesis and increases tail blood flow: effects of pretreatment with 5-HT1A and dopamine D2 antagonists. Neuroscience 154, 1619-1626.

Sanchez, V., O'Shea, E., Saadat, K.S., Elliott, J.M., Colado, M.I., Green, A.R., 2004. Effect of repeated ('binge') dosing of MDMA to rats housed at normal and high temperature on neurotoxic damage to cerebral 5-HT and dopamine neurones. J Psychopharmacol 18, 412-416.

Sanchis-Segura, C., Spanagel, R., 2006. Behavioural assessment of drug reinforcement and addictive features in rodents: an overview. Addict Biol 11, 2-38.

Schenk, S., Gittings, D., Johnstone, M., Daniela, E., 2003. Development, maintenance and temporal pattern of self-administration maintained by ecstasy (MDMA) in rats. Psychopharmacology (Berl) 169, 21-27.

Schenk, S., Hely, L., Lake, B., Daniela, E., Gittings, D., Mash, D.C., 2007. MDMA self-administration in rats: acquisition, progressive ratio responding and serotonin transporter binding. Eur J Neurosci 26, 3229-3236.

Schindler, C.W., Panlilio, L.V., Goldberg, S.R., 2002. Second-order schedules of drug self-administration in animals. Psychopharmacology (Berl) 163, 327-344.

Schmidt, C.J., 1987. Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine. J Pharmacol Exp Ther 240, 1-7.

Schmidt, C.J., Levin, J.A., Lovenberg, W., 1987. In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain. Biochem Pharmacol 36, 747-755.

Schmidt, C.J., Sullivan, C.K., Fadayel, G.M., 1994. Blockade of striatal 5-hydroxytryptamine2 receptors reduces the increase in extracellular concentrations of dopamine produced by the amphetamine analogue 3,4-methylenedioxymethamphetamine. J Neurochem 62, 1382-1389.

Schmidt, C.J., Taylor, V.L., 1987. Depression of rat brain tryptophan hydroxylase activity following the acute administration of methylenedioxymethamphetamine. Biochem Pharmacol 36, 4095-4102.

Schmidt, C.J., Wu, L., Lovenberg, W., 1986. Methylenedioxymethamphetamine: a potentially neurotoxic amphetamine analogue. Eur J Pharmacol 124, 175-178.

Scholey, A.B., Parrott, A.C., Buchanan, T., Heffernan, T.M., Ling, J., Rodgers, J., 2004. Increased intensity of Ecstasy and polydrug usage in the more experienced recreational Ecstasy/MDMA users: a WWW study. Addict Behav 29, 743-752.

Schuster, C.R., Thompson, T., 1969. Self administration of and behavioral dependence on drugs. Annu Rev Pharmacol 9, 483-502.

Sellings, L.H., McQuade, L.E., Clarke, P.B., 2006. Evidence for multiple sites within rat ventral striatum mediating cocaine-conditioned place preference and locomotor activation. J Pharmacol Exp Ther 317, 1178-1187.

Semple, D.M., Ebmeier, K.P., Glabus, M.F., O'Carroll, R.E., Johnstone, E.C., 1999. Reduced in vivo binding to the serotonin transporter in the cerebral cortex of MDMA ('ecstasy') users. Br J Psychiatry 175, 63-69.

Shioda, K., Nisijima, K., Yoshino, T., Kuboshima, K., Iwamura, T., Yui, K., Kato, S., 2008. Risperidone attenuates and reverses hyperthermia induced by 3,4-methylenedioxymethamphetamine (MDMA) in rats. Neurotoxicology 29, 1030-1036.

Siegel, J.M., 1979. Behavioral functions of the reticular formation. Brain Res 180, 69-105.

Spanos, L.J., Yamamoto, B.K., 1989. Acute and subchronic effects of methylenedioxymethamphetamine [(+/-)MDMA] on locomotion and serotonin syndrome behavior in the rat. Pharmacol Biochem Behav 32, 835-840.

Sprague, J.E., Banks, M.L., Cook, V.J., Mills, E.M., 2003. Hypothalamic-pituitary-thyroid axis and sympathetic nervous system involvement in hyperthermia induced by 3,4-methylenedioxymethamphetamine (Ecstasy). J Pharmacol Exp Ther 305, 159-166.

Stefanski, R., Ziolkowska, B., Kusmider, M., Mierzejewski, P., Wyszogrodzka, E., Kolomanska, P., Dziedzicka-Wasylewska, M., Przewlocki, R., Kostowski, W., 2007. Active versus passive cocaine administration: differences in the neuroadaptive changes in the brain dopaminergic system. Brain Res 1157, 1-10.

Stone, D.M., Hanson, G.R., Gibb, J.W., 1987a. Differences in the central serotonergic effects of methylenedioxymethamphetamine (MDMA) in mice and rats. Neuropharmacology 26, 1657-1661.

Stone, D.M., Merchant, K.M., Hanson, G.R., Gibb, J.W., 1987b. Immediate and long-term effects of 3,4-methylenedioxymethamphetamine on serotonin pathways in brain of rat. Neuropharmacology 26, 1677-1683.

Sutoo, D., Akiyama, K., 2004. Music improves dopaminergic neurotransmission: demonstration based on the effect of music on blood pressure regulation. Brain Res 1016, 255-262.

Toukhsati, S.R., Rickard, N.S., Perini, E., Ng, K.T., Gibbs, M.E., 2005. Noradrenaline involvement in the memory-enhancing effects of exposure to a complex rhythm stimulus following discriminated passive avoidance training in the young chick. Behav Brain Res 159, 105-111.

Tsai, H.Y., Lu, Y.H., Wu, C.R., Chen, Y.F., 2005. Effects of noise on monoamine levels in the rat brain using in vivo microdialysis. Pflugers Arch 450, 83-87.

Twining, R.C., Bolan, M., Grigson, P.S., 2009. Yoked delivery of cocaine is aversive and protects against the motivation for drug in rats. Behav Neurosci 123, 913-925.

Tzschentke, T.M., 1998. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Prog Neurobiol 56, 613-672.

Tzschentke, T.M., 2007. Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. Addict Biol 12, 227-462.

Vanderschuren, L.J., Kalivas, P.W., 2000. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology (Berl) 151, 99-120.

Vanhoutte, G., Verhoye, M., Raman, E., Roberts, M., Van der Linden, A., 2002. In-vivo non-invasive study of the thermoregulatory function of the blood vessels in the rat tail using magnetic resonance angiography. NMR Biomed 15, 263-269.

Verheij, M.M., de Mulder, E.L., De Leonibus, E., van Loo, K.M., Cools, A.R., 2008. Rats that differentially respond to cocaine differ in their dopaminergic storage capacity of the nucleus accumbens. J Neurochem.

Verkes, R.J., Gijsman, H.J., Pieters, M.S., Schoemaker, R.C., de Visser, S., Kuijpers, M., Pennings, E.J., de Bruin, D., Van de Wijngaart, G., Van Gerven, J.M., Cohen, A.F., 2001. Cognitive performance and serotonergic function in users of ecstasy. Psychopharmacology (Berl) 153, 196-202.

Verrico, C.D., Miller, G.M., Madras, B.K., 2007. MDMA (Ecstasy) and human dopamine, norepinephrine, and serotonin transporters: implications for MDMA-induced neurotoxicity and treatment. Psychopharmacology (Berl) 189, 489-503.

Volkow, N.D., Fowler, J.S., Wang, G.J., 2002. Role of dopamine in drug reinforcement and addiction in humans: results from imaging studies. Behav Pharmacol 13, 355-366.

Weinberger, N.M.M., T.M. , 1988. Sensitivity of single neurons in auditory cortex to contour: Toward a neurophysiology of music perception. Music Perception 5, 355-390.

Wells, D.L., Graham, L., & Hepper, P.G. , 2002. The influence of auditory stimulation on the behaviour of dogs housed in a rescue shelter. Animal Welfare 11, 385-393.

Wright, A.A., Rivera, J.J., Hulse, S.H., Shyan, M., Neiworth, J.J., 2000. Music perception and octave generalization in rhesus monkeys. J Exp Psychol Gen 129, 291-307.

Wu, S.J., Chou, F.H., 2008. [The effectiveness of music therapy in reducing physiological and psychological anxiety in mechanically ventilated patients]. Hu Li Za Zhi 55, 35-44.

Wu, Y., Jiji, L.M., Lemons, D.E., Weinbaum, S., 1995. A non-uniform three-dimensional perfusion model of rat tail heat transfer. Phys Med Biol 40, 789-806.

Zakharova, E., Leoni, G., Kichko, I., Izenwasser, S., 2009a. Differential effects of methamphetamine and cocaine on conditioned place preference and locomotor activity in adult and adolescent male rats. Behav Brain Res 198, 45-50.

Zakharova, E., Wade, D., Izenwasser, S., 2009b. Sensitivity to cocaine conditioned reward depends on sex and age. Pharmacol Biochem Behav 92, 131-134.

Zakzanis, K.K., Campbell, Z., 2006. Memory impairment in now abstinent MDMA users and continued users: a longitudinal follow-up. Neurology 66, 740-741.

VITA

Allison Anne Feduccia was born on June 22, 1981 in Baton Rouge, LA, the daughter of Michael and Mary Feduccia. She completed her Bachelor of Science (B.S.) degree at Louisiana State University in December 2003 with a major in Psychology and a minor in Biological Sciences. During her undergraduate studies, she participated in a study abroad program where she spent a year studying Biological Psychology at the University of Maastricht in The Netherlands. In the summers of 2003 and 2004, she had an internship doing research in a pharmacology lab at the University of Texas in Austin. She joined the Neuropharmacology Graduate Program at the University of Texas (fall semester 2004) in Dr. Christine L. Duvauchelle's laboratory. While in graduate school she worked as a Teaching Assistant and Graduate Research Assistant. From 2006-2009, she was awarded the Fred M. Jones and Homer L. Bruce Endowed Graduate Fellowship and was appointed to the Alcohol Training Grant (2008-2009). She was a mentor for undergraduate students in the Intellectual Entrepreneurship pre-graduate school internship (2007-2009) and the College of Pharmacy Multicultural Health Research program (2006-2007). Her research focused on the effects of auditory and thermal stimuli on MDMA self-administration and neurochemical responses.

Permanent address (or email): 5927 Riverbend Blvd Baton Rouge, LA 70820

afeduccia@gmail.com

The author, Allison Feduccia, typed this dissertation.