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Combined Effects of MDMA and Ethanol on Locomotor Activity and Place Conditioning in Male and Female Adolescent Sprague-Dawley Rats

Keli A. Herr

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COMBINED EFFECTS OF MDMA AND ETHANOL ON LOCOMOTOR ACTIVITY
AND PLACE CONDITIONING IN MALE AND FEMALE ADOLESCENT
SPRAGUE-DAWLEY RATS

by

Keli A. Herr

A thesis submitted to the Graduate College
in partial fulfillment of the requirements
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June 2014

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Keli A. Herr, M.A.

Western Michigan University, 2014

MDMA, (“Ecstasy”) is commonly abused in combination with ethanol (EtOH). Relatively few preclinical studies have investigated sex differences in animal models of polysubstance use. The current study employed a conditioned place preference (CPP) procedure to assess the behavioral effects of the co-administration of MDMA /EtOH in 32 male and 32 female adolescent Sprague-Dawley rats. Following a 15 min habituation trial, eight 30-min conditioning trials were conducted in two- compartment chambers with different environmental cues. Before each drug conditioning trials, rats were administered intraperitoneal (i.p.) injections of MDMA (6.6 mg/kg), EtOH (1.5 g/kg), MDMA (6.6 mg/kg) and EtOH (1.5 g/kg), or saline. Prior to vehicle conditioning trials, all rats received i.p. saline injections. Place preference was measured by recording time spent in each compartment during a 15 min test session. In both males and females, the hyperlocomotor effects of MDMA and the EtOH/MDMA combination increased with repeated exposure over the course of four conditioning trials. However, there was no evidence for CPP in any treatment group or in either sex. Further research is suggested to determine the influence of age and sex on the abuse liability of MDMA in combination with ethanol and other drugs of abuse.

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Keli Herr

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CHAPTER I

INTRODUCTION

Significant advancements have been made in the last 30 years in pharmacology, neuroscience, and behavioral sciences research that have revolutionized our understanding of sex differences with regard to physiological and behavioral responses to psychoactive drugs. Specifically, research has identified sex differences in brain structures, neural circuits, and neurotransmitters involved in drug actions. Moreover, sex has been identified as an important factor in moderating the effects of various drugs of abuse (Allott & Redman, 2007). Despite numerous reports in the scientific literature since the 1960s regarding notable sex differences in the pharmacokinetics and pharmacodynamics of various drugs in both humans and nonhumans, the majority of published behavioral pharmacology research with nonhumans continues to exclude female subjects (Hughes, 2007). The exclusion of females from preclinical research appears to be based on two primary assumptions. First, earlier investigations involving male and female subjects failed to show differential drug effects (Hughes, 2007). Second, female hormonal fluctuations associated with the estrus cycle could decrease homogeneity and potentially confound drug effects (Carroll & Anker, 2010; Hughes, 2007; & Piper, 2007).

3,4-Methylenedioxymethamphetamine (MDMA) is a popular recreational drug for which differential effects have been reported in males and females. A recent review of preclinical and clinical studies published between 1966 and 2006 revealed substantial evidence for sexually dimorphic patterns in the acute physiological and behavioral effects

of MDMA and possibly the long term consequences of MDMA use (Allot & Redman, 2007). Furthermore, sex-dependent physiological effects of MDMA in rats appear to vary with age and strain. For example, in pubescent Long-Evans rats, males are more sensitive than females to the locomotor stimulant and hyperpyretic effects of MDMA (Koenig et al., 2005), whereas in adult Wistar rats, females appear to be more sensitive to the locomotor stimulant effects (Palenicek et al., 2005). Despite considerable evidence for sex differences in the physiological effects of MDMA, potential differences in the abuse liability of MDMA in males and females have not been systematically explored.

Recreational use of MDMA is popular among adolescents and young adults. In 2012, the National Survey on Drug Use and Health estimated 869,000 adolescents aged 12 to 17 years old had used MDMA, which was similar to the numbers in 2010 (949,000) and 2011 (922,000), but lower than the number reported in 2009 (1.1 million) (NSDUH, 2012). Of particular concern is the common practice of concurrent MDMA use with other drugs of abuse, such as LSD, cocaine, amphetamine, methamphetamine, marijuana, and ethanol (EtOH) (Mohamed, Hamida, Cassel, de Vasconcelos, & Jones, 2011). Scholey et al. (2004) conducted an internet survey to assess polysubstance use patterns in MDMA users and nonusers. Results from 763 individuals suggested that MDMA users reported significantly greater polysubstance abuse than nonusers.

Polysubstance abuse with MDMA increases the risk of cardiovascular toxicity, neurotoxicity, and fatal overdose (Schifano, 2004; Mohamed et al., 2011). In consideration of the popularity of polysubstance abuse associated with MDMA, preclinical research is especially relevant to understanding the potential risks of concurrent exposure to MDMA with other abused substances. However, only a few

preclinical studies have investigated the combined behavioral effects of MDMA and other drugs of abuse, and most of these studies have utilized only male rodents.

The following sections provide a brief history of MDMA and outline the acute physiological and behavioral actions and pharmacokinetics of MDMA. Next, studies on sex differences in the effects of MDMA alone, and in combination with other drugs, are addressed. Finally, the conditioned place preference (CPP) procedure is described as an animal model predictive of abuse liability and previous research utilizing this procedure to assess MDMA in combination with other abused substances is addressed.

MDMA

MDMA is a structural analog of the central nervous system (CNS) stimulant, amphetamine and the psychedelic drug, mescaline. MDMA was first synthesized by Merck in 1912 as a potential blood clotting agent (Shulgin, 1986), but its CNS effects were not thoroughly examined for several decades. In 1953, the United States Army funded toxicological studies on MDMA and other mescaline analogs. This research was declassified and published in 1973 by Hardman, Haavik, and Seevers. The first study reporting MDMA having psychoactive properties in humans was published by Shulgin and Nichols in 1978 (Shulgin, 1986). Following the discovery of MDMA's psychoactive properties, it was profusely used by psychotherapists as a therapeutic tool. Therapists utilized MDMA because its effects of producing feelings of well-being and trust were seen as a way to enhance the therapeutic relationship between patient and psychotherapist (Shulgin, 1986). In 1985, the Drug Enforcement Administration classified MDMA as a Schedule I drug due to high abuse potential and the lack of sufficient evidence to support its clinical use (Green, Mechan, Elliott, O'Shea, & Colado, 2003).

Despite the negative consequences of MDMA abuse, recreational use gained popularity, especially among young adults. MDMA's street name is "Ecstasy" and it is typically sold in tablet or powder form. However, street samples of "Ecstasy" are frequently not pure MDMA, but mixtures of various psychostimulants (Green et al., 2003). The typical routes of administration include oral or intranasal. The onset of MDMA's effects are typically experienced within 30-60 minutes after ingestion and last approximately 3-5 hours. MDMA's psychoactive effects in humans include euphoria, increased sexual arousal, enhanced empathy toward others, relaxation, sense of closeness, intensity of sensations and perceptions, and occasionally hallucinations. In rats, the most noted observable behaviors are side-to-side head movements, front paws moving from side-to-side, and low body posture. In both human and animals, MDMA increases heart rate, blood pressure, and body temperature (Green et al., 2003).

MDMA's chemical properties and pharmacological effects are unique. As noted above, MDMA is an amphetamine derivative with a ring substitution that modifies its pharmacological properties and accounts for its CNS stimulant effects (Kalant, 2001). MDMA differs from amphetamine because of the methylenedioxy (-O-CH₂-O-) group attached to it, resembling the structure of mescaline, a hallucinogenic drug (Green et al., 2003). Some researchers in the scientific community have classified MDMA as an 'entactogen' or 'empathogen' because of its ability to produce mild euphoria, well-being, and an enhanced sense of insight and connectedness with others (Green, 2003). Thus, MDMA's unique effects are distinguishable from other psychostimulants and hallucinogens.

MDMA's neurochemical mechanisms of action involve increased extracellular serotonin (5-HT), dopamine (DA), and norepinephrine (NE) (Kalant, 2001). The elevation in monoamines may result from a combination of increased release and altered reuptake of 5-HT and DA. In addition, MDMA has a high affinity for the serotonin transporter (SERT) and there are indications that MDMA may reverse SERT activity causing additional serotonin release. MDMA also inhibits the activity of monoamine oxidase (Kalant, 2001). Lastly, MDMA has been reported to elevate plasma prolactin, oxytocin, and cortisol/corticosterone (Cole, Sumnall, O'Shea, & Marsden, 2003). Areas in the brain primarily affected by MDMA include the neocortex and limbic brain regions responsible for mediating mood, cognition, sensory, and perceptual processes (Kalant, 2001).

The pharmacokinetics of MDMA have been identified and thoroughly characterized in adults (human and animal). MDMA crosses the blood-brain barrier within 15-25 minutes after oral administration (Kalant, 2001). MDMA then reaches peak plasma levels in approximately two hours. MDMA is metabolized mainly by the enzyme CYP2D6 found in the liver. Elimination of MDMA is slow; the half-life is approximately 8-9 hours in humans and two-hours in rats (Kalant, 2001).

MDMA is commonly administered with other drugs, presumably to enhance and/or counteract their effects. Perhaps the most common drug used concurrently with MDMA is EtOH because it's legal, easily obtainable, and socially acceptable (Mohamed et al., 2011). Among recreational drug users, MDMA is reportedly used in combination with EtOH because it prolongs the euphoric effects of MDMA (Mohamed, Hamida, de Vasconcelos, Cassel, & Jones, 2009). EtOH is one of the oldest known psychoactive

drugs used by humans. It is classified as a CNS depressant and is an indirect agonist for gamma-amino-butyric acid (GABA) (Tomkins & Sellers, 2001). EtOH can suppress activity in brain pathways associated with the control of movement, speech, judgment, and memory (Mohamed et al., 2011). In recent years, researchers have begun investigating the effects of the co-administration of MDMA and EtOH using animal models.

Research using nonhumans has indicated that EtOH enhances the behavioral effects of MDMA and attenuates the risk of MDMA-induced elevated body temperature in rats (Cassel, Jeltsch, Koenig, & Jones, 2004; Cassel, Hamida, & Jones, 2007; Hamida, Bach, Plute, Jones, Kelche, & Cassel, 2006; Hamida, Plute, Cosquer, Kelche, Jones, & Cassel, 2008; Mohamed et al., 2009). For example, a study conducted by Cassel et al., (2004) investigated the effects of the combination of EtOH and MDMA on locomotor activity and core body temperature in rats. Thirty-five male Long-Evans rats aged 3 months were injected intraperitoneally (i.p.) with one of the following: 1.5 g/kg EtOH, 10mg/kg MDMA, 7.5 mg/kg MDMA and 1.5 g/kg EtOH, or saline. Locomotor activity and body temperature were measured for six hours on four consecutive days. The results indicated that the combination of MDMA and EtOH increased locomotor activity on all four days. In addition, EtOH decreased MDMA-induced hyperthermia on the first day of treatment, but not on the other three days. Thus, EtOH enhanced the locomotor stimulant effects and blocked the acute effects of elevated body temperature induced by MDMA in rats (2004).

Another study conducted by Hamida et al. (2007) investigated the combination of EtOH and MDMA administered intermittently on four occasions to determine whether

tolerance or sensitization occurred. The rationale for using intermittent administration of MDMA and EtOH was that it represented recreational MDMA use in humans. Seventy-nine male Long-Evans rats aged 3 months were injected i.p. with one of the following in a volume of 7.5 ml/kg: 1.5 g/kg EtOH, 6.6 mg/kg MDMA, 6.6 mg/kg MDMA and 1.5 g/kg EtOH, or saline. Locomotor activity was measured for two hours before and two hours after treatment on days 4, 6, 11, and 13 of the experiment. Body temperature was also measured 60 minutes prior to and 60 minutes after each injection. An additional 10 rats were used (same strain and age as above) to measure MDMA and MDA concentrations after i.p. injection of 10 mg/kg of MDMA (n=5) or the combination of 10 mg/kg of MDMA and 1.5 g/kg of EtOH (n=5). Forty-five minutes after the injections, the rats were euthanized and the hippocampus, frontoparietal cortex, and striatum were removed and processed to determine brain levels of MDMA and MDA.

Results suggested that intermittent administration of the combination of MDMA and EtOH produced partial protection against MDMA-induced hyperthermia by EtOH and gradual development of sensitization to locomotor activating effects of this drug combination (Hamida et al., 2007). The ratio of MDA to MDMA (about -16%) was lower in the brains of rats administered the combination compared to those administered only MDMA. These results suggest that the combination of EtOH alters the brain distribution of MDMA and metabolism to MDA (2007).

Sex Differences

Recent evidence from both animal and clinical studies has indicated that males and females differ in pharmacokinetics, pharmacodynamics, and behavioral responses to abused substances, such as alcohol, MDMA, psychostimulants, and nicotine (Allott & Redman, 2007; Becker & Hu, 2007). However, despite this evidence, the vast majority of research on psychoactive drugs continues to use only male subjects and many findings are generalized to both male and female populations (Becker & Hu, 2007; Hughes, 2007).

Hughes (2007) conducted a survey of various journal articles to determine the number of rodent studies published using male only, female only, or both sexes during a 20-month period (February 2005- September 2006). Results indicated that 80 to 90% of studies used only male rodents as subjects. The exclusion of female rodents in psychopharmacology research is due primarily to concerns about the impact of variability in the female's estrous cycle on behavioral measures (Hughes, 2007). Previous research on the behavioral effects of psychoactive drugs on the estrous cycle has utilized psychostimulants, such as cocaine and amphetamine (Becker & Hu, 2007). Evidence from animal studies on the behavioral effects of these drugs across the estrous cycle have indicated that estrogen facilitates drug-seeking and using behaviors and progesterone reduces behaviors associated with the aversive effects (Becker & Hu, 2007).

Several studies have assessed the influence of estrogen and progesterone on the locomotor effects of psychostimulants, particularly cocaine and amphetamine (Becker, & Hu, 2007; Carroll & Lynch, 2000; Sell, Scalzitti, Thomas, & Cunningham, 2000; Zhou, Cunningham, and Thomas, 2003). For example, Sell et al. (2000) investigated the role of ovarian hormones and the estrous cycle on locomotor activity produced by cocaine in male and female SD rats. A series of experiments were conducted to determine the

behavioral effects of cocaine in male and female rats and interactions with the estrous cycle in females. Adult male rats were only used as comparisons in the first experiment to investigate sex differences on locomotor activity. Rats were given an i.p. injection of either saline or 10 or 15 mg/kg of cocaine. Results indicated that females were significantly more sensitive than males to the locomotor activating effects of cocaine. To determine the effects of ovarian hormones and the estrous cycle on cocaine-induced activity, the researchers used only females as subjects in the next series of experiments. Female rats were ovariectomized (OVX) and implanted with a hormone capsules of either estrogen, progesterone, or estrogen and progesterone. Rats were administered 5.0 mg/kg of cocaine and locomotor activity was measured for 120 minutes. Results indicated that OVX rats with either estrogen or estrogen and progesterone, but not progesterone alone, exhibited increased horizontal and vertical activity responses to cocaine than OVX rats. Thus, ovarian hormones, such as estrogen enhanced behavioral responsiveness to cocaine.

In a related study, Zhou, et al (2003) conducted two experiments to determine the influence of estrogen on the locomotor effects of MDMA and cocaine in adult SD females. All of the females (128) were ovariectomized (OVX) and half received estrogen (OVX + E) implants. In the first experiment, 64 female rats were administered either saline or 1, 2, or 4 mg/kg MDMA i.p. and locomotor activity was measured for 120 minutes. In experiment two, 64 female rats were administered either saline or 5, 10, or 20 mg/kg cocaine i.p. and locomotor activity was measured for 120 minutes. Results indicated enhanced locomotor activity in both MDMA and cocaine OVX + E rats

compared to OVX rats. Thus, estrogen enhanced sensitivity to the effects of MDMA and cocaine (Zhou, et al., 2003).

Several animal studies have also established sex differences in the abuse-related behavioral effects of psychoactive drugs. For example, Lynch and Carroll (2000) investigated sex differences in the self-administration of cocaine in eight adult male and eight adult female Sprague Dawley (SD) rats. They trained rats to self-administer 0.2 mg/kg of cocaine under a fixed-ratio (FR) 1 schedule of reinforcement and implemented a priming model to extinguish cocaine reinforcement by replacing it with saline infusions. After responding was extinguished, rats were administered priming injections of saline, or 0.32, 1.0, and 3.2 mg/kg cocaine. Results indicated that female rats self-administered significantly more saline infusions after priming injections of 1.0 mg/kg and 3.2 mg/kg cocaine compared to males. Thus, female rats appear to be more sensitive than males to relapse factors associated with cocaine (Lynch & Carroll, 2000).

Few studies have investigated the influence of sex differences on the behavioral and biological responses to MDMA. Previous research suggests that at least three possible explanations for sex differences in MDMA's effects (Allott & Redman, 2007). The first explanation relates to the influence of estrogen and progesterone in regulating MDMA effects in females. Second, pharmacokinetics processes, such as metabolism and distribution may influence the distribution and bioavailability of MDMA. This may aid in understanding why males are more sensitive to the toxicity of MDMA than females. Lastly, the existence of sex differences in brain structures, neurotransmitter systems and functions could account for sex differences in behavioral responses to MDMA (Allott & Redman, 2007).

One of the first studies to report sex differences in MDMA's effects was conducted by McNamara, Kelly and Leonard (1995). The authors investigated the acute effects of a single 20 mg/kg dose of MDMA on brain monoamine levels, body temperature, and serum corticosterone in adult male (200-250 g) and female (150-180 g) SD rats. Results suggested that males and females differ in body temperature, activity levels, serum corticosterone, 5-HT, DA, and NA levels, and metabolites, 5-HIAA and DOPAC (1995). However, a limitation to this study was the differences reported were not consistently associated with either males or females across the different measures, thus making interpretation difficult. Fonsart et al. (2008) determined the median lethal dose (LD_{50}) of MDMA in male and female rats. Twenty male and female SD rats approximately 7-9 weeks old were given a single subcutaneous injection of MDMA (5-40 mg/kg). Results suggested that the LD_{50} for MDMA was significantly lower in male than female rats. These findings indicate males are more sensitive than females to MDMA's acute toxicities (Fonsart et al., 2008).

With the exception of a few studies, the majority of studies on sex differences associated with MDMA's effects have assessed locomotor activity in rodents. Walker, Williams, Jotwani, Waller, Francis, and Kuhn (2007) investigated the effects of MDMA on locomotor activity, body temperature, performance in an elevated plus maze, and brain serotonin levels in adult male and female SD rats. All rats received either saline or 15 mg/kg i.p. injections for four days. The authors used separate cohorts of rats to measure the effects of MDMA on each of the four dependent variables. Results suggested that females were more sensitive to the locomotor effects of MDMA than males after four days and after a two-week washout period. Similar results in elevations

of temperature were reported for both males and females. Additionally, MDMA- treated males and females spent less time in the open arms of the elevated plus maze. Thus, females showed greater locomotor activity compared to males, but MDMA produced similar effects on body temperature and anxiety measures in both sexes (Walker et al., 2007).

Koenig et al. (2007) investigated the effects of MDMA on locomotor activity in adolescent male and female Long-Evans rats (PND 39). Rats received an i.p, injection of 10 mg/kg MDMA three times every two hours and locomotor activity was measured. The results indicated after the second injection, locomotor activity was enhanced in the males but not in the females and after the third injection all the males and only three females died. Thus, adolescent male rats are more sensitive to the locomotor and neurotoxic effects of MDMA than females (Koenig et al., 2007).

To date, the majority of published nonhuman studies on sex differences associated with MDMA have utilized fairly high doses and measured physiological responses and locomotor activity. Animal models have not been widely used to explore sex differences in the abuse liability of MDMA. The next section summarizes the use of conditioned place preference as an assay of abuse liability.

Conditioned Place Preference

Conditioned place preference (CPP) is a popular paradigm used to investigate the neural and behavioral mechanisms underlying the rewarding and/or aversive properties of abused drugs (Prus, James, & Rosecrans, 2009). Specifically, CPP is designed to predict conditioned rewarding effects of abused substances through a Pavlovian conditioning process involving explicit pairing of environmental stimuli or context with the presence

of a drug. Following repeated pairings of a distinct environmental context with drug-induced stimuli and pairings of an alternative environmental context with the absence of drug-induced stimuli, the amount of time an animal spends in each environmental context during a subsequent test session is used as an index of the conditioned rewarding effects of the drug. It is well documented that most addictive drugs establish CPP in rodents (Bardo & Bevins, 2000). Therefore, CPP is commonly utilized as a preclinical behavioral assay of drug abuse liability to screen novel compounds.

Since 1976, a number of studies utilizing the CPP have been published as a behavioral assay investigating psychoactive drugs. Typically, the apparatus is setup to have two or three compartments. In the three-compartment apparatus a middle compartment serves as a neutral-divider between the two distantly different outer compartments (Prus et al., 2009). In the two-compartment assay, the compartments are separated by a door that is open during habituation and the test phase and shut during conditioning days. Each of the two compartments differs in their environment contextual cues. For example, the environments may differ in their tactile, olfactory, or visual cues, such as one environment has walls with horizontal black and white stripes and the other environment has walls with vertical black and white stripes.

Although studies differ along methodological dimensions, the overall basic process of conditioning is the same. A plethora of research has been published utilizing the CPP assay, and overall several drugs of abuse have shown to establish a place preference, including cocaine, amphetamine, heroin, morphine, alcohol, nicotine, and MDMA (Cole, Sumnall, O'Shea, & Marsden, 2003; Prus et al., 2009). In a recent study, Panos and Baker (2012) examined combinations of MDMA and cocaine using a CPP

procedure. Results suggested that low MDMA doses (1.5, 3.0 mg/kg) produced only modest effects on locomotor activity and did not establish CPP, but appeared to enhance the locomotor and conditioned rewarding effects of 20 mg/kg cocaine (Panos & Baker, 2012).

To date, relatively few studies have investigated the effects of the combination of MDMA and EtOH on CPP. Jones et al. (2011) investigated the effects of the combination of MDMA and EtOH using a CPP procedure. Twenty-seven adult male Long-Evens rats were i.p. injected with either EtOH (0.75 g/kg), MDMA (6.6 mg/kg), EtOH (0.75 g/kg) + MDMA (6.6 mg/kg), or saline and CPP was assessed. Results suggested that only the combination of MDMA and EtOH produced CPP in male Long-Evens rats. Based on their findings, the authors concluded that EtOH may increase the risk for compulsive MDMA use and augment the risk for dependence. They also suggested this risk may depend on the particular dose of EtOH and noted that this issue requires further study (Jones et al., 2011).

It is well established that women and men can respond differently to some psychoactive drugs. The reasons for these differences are not well understood. Currently, there is a paucity of preclinical research comparing behavioral responses and abuse liability in males and females. Although several studies have examined the effects of MDMA in combination with other abused substances, these studies used male-only subjects. There are currently no published reports on the combined effects of EtOH/MDMA comparing males and females. The objective of this study was to investigate the combination of EtOH/MDMA in adolescent male and female Sprague-Dawley rats using the CPP assay.

CHAPTER II

METHODS

Subjects

Thirty-two male and 32 female adolescent (PND=28) Sprague-Dawley rats (Charles River Laboratories, Portage, MI) weighing approximately 50-90 grams were used as subjects. All rats were individually housed in polycarbonate cages in a colony maintained on a 12:12 hour light/dark cycle (Lights on 7AM-7PM). The animal colony was maintained at a constant temperature (20-22°C) and at constant humidity levels (50-60%). Food and water were available *ad libitum* in the animals' home cages throughout the study. Rats of each sex were randomly assigned to one of four treatment groups (saline, MDMA, EtOH, MDMA/EtOH) for place conditioning experiments. The animal use protocol was approved by the Institutional Animal Care and Use Committee of Western Michigan University.

Apparatus

The CPP procedure utilized in this study was a biased two-compartment procedure. Conditioned place preference procedures were conducted simultaneously in eight identical custom designed Plexiglas chambers (40.5 cm L×40.5 cmW×40.5 cm H). Each compartment contained distinct contextual (tactile and visual cues). One side had vertical black and white stripes walls with textured plastic floors and the other side had horizontal black and white stripes walls with aluminum floors. A removable door separated the two compartments. Each chamber was housed within an Accuscan automated activity monitoring system (Accuscan instruments, Inc., Columbus, OH) equipped with infrared emitters and detectors connected to a microprocessor. Locomotor

activity and time spent in each side of chamber were processed using Versmax software (Accuscan instruments, Inc., Columbus, OH).

Drugs

MDMA was obtained from the National Institute on Drug Abuse (Rockville, MD). MDMA was dissolved in 0.9% sterile saline at a concentration of 0.88 mg/ml and injected intraperitoneally (i.p.) at an injection volume of 7.5 ml per kg, to produce a dose of 6.6 mg/kg. Ethanol (100%) was purchased from Aaper Alcohol (Shelbyville, KY) and diluted to 10% in 0.9% saline and injected at a volume of 7.5 ml per kg to produce a dose of 0.75 g/kg. For the combination, MDMA (6.6 mg/kg) was dissolved in EtOH (0.75 g/kg) solution. This dosing regimen was selected based on previously published research (Jones et al., 2010).

Procedures

The CPP procedure consisted of three distinct phases: habituation, conditioning, and post-conditioning. Habituation consisted of one, 15-minute session on day 1. During habituation, the door in the center wall was removed, thus allowing the rats to have access to both compartments. The purpose of the habituation phase was to assess time spent in each compartment to determine if there was any pre-conditioning bias. The least-preferred compartment for each subject was paired with drug during conditioning. Conditioning commenced on the next day following habituation and thereafter for eight consecutive days. On days 2, 4, 6 and 8, saline, MDMA, EtOH, or MDMA + EtOH was administered immediately before confinement to the drug-paired compartment for 30 minutes. On days 3,5,7, and 9 all groups were given an injection of saline and confined to the opposite compartment (non-drug side). Locomotor activity was recorded during all

conditioning trials. On day 10, the post-conditioning test was conducted. Rats received no injections and were placed in the chamber with the doors removed and allowed to roam freely for 15 minutes. Locomotor activity and time spent in each compartment was recorded. A difference score was calculated to assess evidence for CPP; the time spent in vehicle-paired side was subtracted from the time spent in the drug-paired side.

Statistical Analysis

All statistical analyses and graphs were conducted using SPSS (IBM Armonk, New York) and GraphPad Prism 4.0 (San Diego, CA) software. A repeated measures general linear model was conducted on horizontal activity during all conditioning trials with sex and treatment group as the between subjects factor and conditioning trial as a within subjects factor. Additionally, averages were calculated for the four conditioning trials and one way analyses of variance (ANOVA) for each sex were conducted to compare the four-day drug averages among the four treatment groups. Subsequently, a two-way ANOVA was conducted to compare males and females four-day drug averages. CPP test results were analyzed using a one-way ANOVA for each sex and a two-way ANOVA comparing sex difference. CPP test results were conveyed as a difference score, which was calculated by subtracting the time spent in the saline-paired side from the time spent in drug-paired side. Bonferroni post-hoc tests were conducted for significant differences between specific treatment groups. Results were considered statistically significant if $p < 0.05$.

CHAPTER III

RESULTS

Concurrent treatment with 6.6 mg/kg MDMA and 0.75 g/kg ethanol increased activity in both male and female adolescent rats relative to their respective controls, and this effect appeared to be more pronounced in males. Figure 1 displays group differences in horizontal activity during the four 30-minute drug conditioning trials in males (left) and females (right). Statistical analysis of these data using a general linear model (Sex x Treatment Group x Drug Conditioning Trial) with repeated measures on Conditioning Trial revealed a statistically significant treatment group effect [$F(3,55) = 6.081$, $p < 0.001$]. Although there appeared to be a progressive increase in activity from drug trial day 1 to drug trial day 4 in MDMA-treated rats, there was no statistically significant main effect of drug conditioning trial on activity [$F(3,165) = 4.939$, $p > 0.05$]. There was also no statistically significant effect of sex ($F(1,55) = 1.608$, $p > 0.05$) and there were no significant interactions between drug conditioning trial and sex [$F(3,165) = 0.252$, $p > 0.05$], between sex and treatment group [$F(3,55) = 0.820$, $p > 0.05$], or three way interaction among drug conditioning day, sex, and treatment group [$F(3,165) = 4.939$, $p > 0.05$].

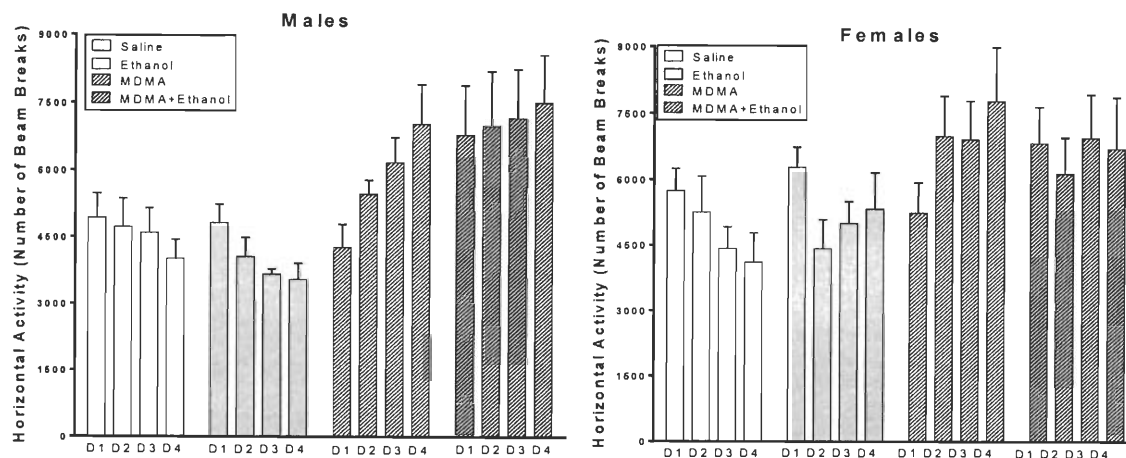


Figure 1. Mean (\pm S.E.M.) horizontal activity of males (left) and females (right) during four drug conditioning trials for each treatment group.

Since there were no statistically significant differences in activity over the four drug conditioning trials, averages were calculated for the four drug conditioning trials and these data were analyzed using a two way ANOVA (sex x treatment group). Figure 2 depicts the average horizontal activity for the four drug conditioning trials in males (left) and females (right). Again, there was a significant main effect of treatment group, [$F(3, 55) = 6.32, p < 0.001$]. However, there was no statistically significant main effect of sex nor was the sex x treatment group interaction statistically significant. Bonferroni multiple comparison tests showed significant differences among treatment groups only in the males. Specifically, horizontal activity in EtOH+MDMA treated males was significantly higher than in saline-treated males ($p < 0.05$) or EtOH-treated males ($p < 0.05$), but not significantly greater than MDMA treated males ($p > 0.05$). In females, MDMA and EtOH+MDMA produced slightly higher levels of activity than either saline or EtOH alone, however these differences were not statistically significant.

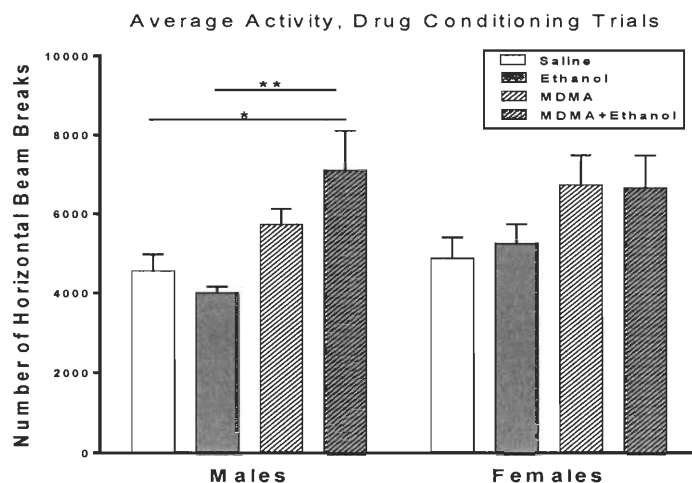


Figure 2. Averages of horizontal activity in males and females during four 30 min drug conditioning trials.

Figure 3 depicts the CPP test results expressed as the mean (\pm S.E.M.) difference scores determined by time spent in each compartment by males (left) and females (right) following conditioning trials with saline, MDMA, EtOH, or the MDMA+EtOH mixture. In both males and females, none of the drug-treated groups spent more time in the drug-paired environment compared to the vehicle-control group during the 15 min CPP test. A one-way ANOVA on the difference scores revealed no statistically significant evidence for conditioned place preference in either males [$F(3,31) = 0.6282, p > 0.05$] or females [$F(3,30) = 0.3821, p > 0.05$].

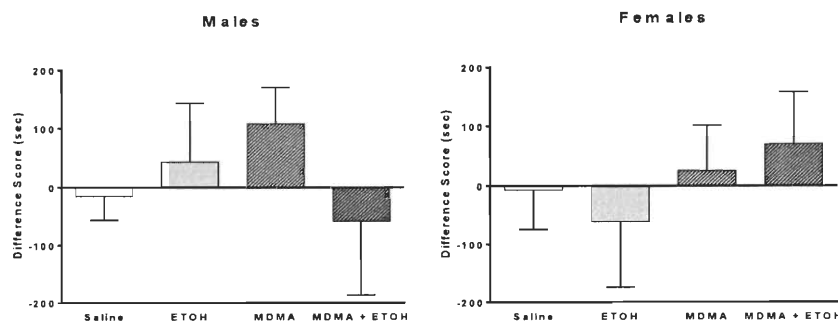


Figure 3. Mean (\pm S.E.M.) difference score (time spent in drug compartment – time spent in saline compartment).

CHAPTER IV

DISCUSSION

The purpose of the present experiment was two-fold. First, this study assessed the locomotor and conditioned behavioral effects of the co-administration of MDMA+EtOH in comparison to each drug alone. Second, sex differences were examined with regard to the effects of these substances. The primary findings suggested that the co-administration of 6.6 mg/kg MDMA+0.75 g/kg EtOH failed to establish a conditioned place preference in either male or female adolescent SD rats following four drug conditioning trials. Additionally, the results of the current study failed to find evidence for sex differences following the co-administration of MDMA+EtOH. However, a significant main effect was found among treatment groups in males, but not females. The results, limitations, and directions for future research are discussed in further detail below.

Results of the current study indicate that total horizontal activity during four 30 minute drug conditioning trials differed in male and female adolescent SD rats. In males administered the combination of MDMA+EtOH, activity was significantly higher than that of saline- or EtOH-treated males, but not significantly higher than MDMA-treated males. In females, MDMA and MDMA+EtOH produced slightly higher activity than saline or EtOH alone, but these differences were not statistically significant. Additional studies, with varying doses of MDMA+EtOH will be needed to better understand the differences between males and females adolescent rats in the locomotor effects of these substances.

In both sexes, the hyperlocomotor effects of MDMA and the combination of MDMA+EtOH increased with repeated exposure over the course of four 30 minute drug conditioning days. Visual analysis of horizontal activity for each of the drug conditioning days plotted into 5 minute intervals suggests a trend towards behavioral sensitization. These findings are consistent with previous research suggesting that the combination of EtOH (1.5 g/kg) /MDMA (6.6 mg/kg) produced behavioral sensitization in adult male rats (Hamida, et al., 2008). Behavioral sensitization is characterized by a progressively increased response following repetitive drug administration and is an established preclinical index of drug dependence risk (Robinson and Becker, 1986). However, an important measure of locomotor activity that typically is used to investigate sensitization, vertical activity, was not measured in the present study due to constraints of the CPP apparatus. Whereas this study was not specifically designed to assess behavioral sensitization, future studies aimed at assessing the development of sensitization with repeated concurrent exposure to MDMA and EtOH should assess multiple measures of ambulatory activity.

The current findings indicated that the combination of EtOH (1.5 g/kg) and MDMA (6.6 mg/kg) failed to establish conditioned place preference in male or female adolescent SD rats. These results are inconsistent with previous research using similar dose combinations of MDMA and EtOH that established a CPP in adult male Long-Evans rats (Jones et al., 2011). Several explanations could account for the discrepancies in the results. First, different strains of rats were used. The current study used Sprague-Dawley rats compared to Jones et al. (2011) using Long-Evans rats.

Secondly, the ages of rats used were different. For instance, the current study investigated combination of MDMA+EtOH using adolescent rats, whereas Jones et al. (2011) assessed adult rats. Thus, the influence of age may have contributed to developmental differences in learning, which may have influenced the extent to which animals learned to successfully discriminate vehicle- and drug-paired cues (Izenwasser, 2005). Specifically, it is known that many brain regions and neurotransmitters that are involved in associative and contextual learning are undergoing maturational and structural changes in adolescence. The human and rat brain undergo structural and neurochemical maturation throughout adolescence. At the structural level, myelination of connections from the prefrontal cortex (PFC) to limbic structures increases during adolescence, which is involved in higher-order cognitive functioning, such as cortical processing and working memory (Izenwasser, 2005). Additionally, mesolimbic dopamine (DA) pathways undergo substantial development of the receptors during adolescence (2005).

Aberg, Wade, Wall, and Izenwasser (2007) investigated the effects of MDMA on cocaine CPP in adult (PND 60) and adolescent (PND 33) SD rats. Each rat was administered either 2 or 5 mg/kg MDMA or vehicle for seven days and locomotor activity was measured. Following the seventh day, there was a five day washout period then assessment of cocaine-CPP commenced. For three days, all the rats were given vehicle in the morning and 10 mg/kg of cocaine in the afternoon in 30 minute trials. Then on the fourth day, all the rats were tested for CPP. Results suggested that cocaine produced a CPP in adolescent rats pretreated with MDMA, but not in the adults pretreated with MDMA. Additionally, sensitization to 2 mg/kg MDMA occurred in

adults, but not in adolescents and for both adults and adolescent rats sensitization occurred following treatment with 5 mg/kg MDMA (Aberg et al., 2007). These findings suggested that there are differential behavioral effects in adult and adolescent rats following repeated administration of MDMA and increased rewarding effects of cocaine in adolescent rats compared to adults.

To our knowledge, this is the first study analyzing sex differences on the effects of concurrent treatment with MDMA and EtOH in male and female adolescent SD rats. This is surprising considering the research evidence supporting the hypothesis that there are sex differences in drug responses and the involvement of gonadal hormones. However, the use of pre-pubescent SD rats could explain the lack of differences between males and females in the current study. Future research is warranted to further investigate and explain sex differences in behavioral responses to the co-administration of MDMA with other abused drugs. This would require future studies to routinely include females and males as subjects and sex as an independent variable in data analysis. The majority of nonhuman behavioral pharmacology studies are still conducted using only adult male rats, thus raising questions concerning errors about the generalization of research results to females. Hormonal fluctuations in females that may affect pharmacokinetics, pharmacodynamics, and efficacy are poorly understood. Further investigations should focus on emphasizing the importance of inclusion variables, such as age, sex, and gonadal hormones, on the behavioral mechanisms underlying MDMA in combination with other drugs.

In summary, the current study examined the locomotor conditioned rewarding effects of concurrent repeated exposure to MDMA+EtOH in male and female adolescent

rats. Including females as subjects in research could lead to more effective and efficient ways of addressing drug abuse prevention approaches and lead to a better understanding of sex-specific consequences of abused drugs.

APPENDIX

Institutional Animal Care and Use Committee
(IACUC) Protocol Clearance

WESTERN MICHIGAN UNIVERSITY

Institutional Animal Care and Use Committee



Date: March 10, 2010

To: Lisa Baker, Principal Investigator

From: Robert Eversole, Chair

A handwritten signature in black ink, appearing to read "RE", is written over the "From" line.

Re: IACUC Protocol No. 10-02-05

Your protocol titled "Conditioned Place Preference Procedures in Rats" has received approval from the Institutional Animal Care and Use Committee. The conditions and duration of this approval are specified in the Policies of Western Michigan University. You may now begin to implement the research as described in the application.

The Board wishes you success in the pursuit of your research goals.

Approval Termination: March 10, 2011

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