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Sex Differences in Serotonergic and Dopaminergic Mediation of LSD Discrimination in Rats

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SEX DIFFERENCES IN SEROTONERGIC AND DOPAMINERGIC MEDIATION OF LSD DISCRIMINATION IN RATS

by

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A dissertation submitted to the Graduate College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Psychology
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SEX DIFFERENCES IN SEROTONERGIC AND DOPAMINERGIC MEDIATION OF LSD DISCRIMINATION IN RATS

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After decades of opposition, a resurgence of interest in the psychotherapeutic potential of LSD is gaining acceptance in the medical community. Future acceptance of LSD as a psychotherapeutic adjuvant may be predicated on knowledge about its neural mechanisms of action. Preclinical drug discrimination assay offers an invaluable model to determine the neural mechanisms underlying LSD’s interoceptive stimulus effects. Unfortunately, current preclinical literature on LSD discrimination is based on results obtained exclusively in male subjects. The present study represents the first known preclinical assessment of possible sex differences in the discriminative stimulus effects of LSD.

Adult female (n=8) and male (n=8) Sprague-Dawley rats were trained to discriminate 0.08 mg/kg LSD from saline under a fixed ratio 20 schedule of food reinforcement. Once discrimination was established, substitution tests were conducted with other hallucinogens (mescaline, DOM, psilocybin), mixed psychedelic-stimulants (MDMA, (+)-MDMA, (-)-MDMA, (+)-MDA, (-)-MDA), synthetic cathinones (MDPV, mephedrone) and psychostimulants (cocaine, amphetamine). Antagonist tests were conducted with serotoninergic antagonists (WAY 100,635, MDL 100,907, pirenperone) and dopaminergic antagonists (haloperidol, SCH 23390).
Stimulus substitution results indicate higher levels of LSD-substitution with other serotonergic hallucinogens in females compared to males and some evidence for sex differences in the level of partial substitution by synthetic cathinones and the enantiomers of MDMA and MDA. Specifically, greater partial substitution was observed with (±)-MDMA, (+)MDMA, and (+)-MDA, in males and greater partial substitution was observed with (-)-MDMA, (-)-MDA, MDPV, and 4-MMC in females. Dopamine antagonists failed to block LSD in either males or females, but had stronger rate suppressant effects in males. The 5-HT2A antagonist, MDL 100,907 blocked LSD discrimination in both males and females, although complete blockade was evident at lower doses in males. These results suggest the relative contribution of serotonergic versus dopaminergic activity to the LSD cue varies between males and females. These findings may be informative for future investigations with human populations regarding possible sex differences in the subjective effects of LSD.
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CHAPTER I
INTRODUCTION

Lysergic acid diethylamide (LSD) is one of the most potent and safest psychoactive chemicals known to mankind. After decades of opposition, a resurgence of interest in the psychotherapeutic potential of LSD is now evident in the clinical psychopharmacology literature. Clinical trials are currently in progress with LSD as an adjuvant to psychotherapy in the treatment of anxiety, depression, and drug addiction (Carhart-Harris, et al., 2016a). Increased acceptance of LSD as a tool in psychotherapeutic settings could facilitate further discovery of the underlying neural mechanisms involved in its unique psychoactive properties.

Undoubtedly, preclinical research utilizing animal models has proven invaluable in discerning the neurochemical actions of psychoactive drugs. In particular, the drug discrimination paradigm has contributed considerable knowledge about the neurochemical substrates underlying the interoceptive stimulus effects of many drugs, including those of LSD. Nevertheless, the precise cellular mechanisms responsible for LSD-induced hallucinations are still not entirely understood after nearly 50 years of preclinical psychopharmacology research. Furthermore, there are currently no known published studies regarding possible sex differences in the discriminative stimulus effects of LSD. In fact, despite multiple calls to action to evaluate sex as an important biological variable, most preclinical behavioral pharmacology studies continue to use only male subjects. The National Institute of Health (NIH) and other major research institutes have made recent steps in addressing the sex bias in preclinical and clinical research. The primary aim of the current study was to ascertain if the psychopharmacological profile of LSD varies between male and female Sprague-Dawley rats. The following introduction
will first address the relevance of sex as a biological variable in psychopharmacology research in support of the rationale for the current study. The remainder of the introduction will provide a historical background and broad overview of current knowledge regarding the psychopharmacology of LSD.

**Sex as a Biological Variable**

Preclinical research is invaluable for the advancement of the scientific field, especially in translating to the clinical population. Much of our understanding of the mechanisms of drug action, behavioral responses to drugs, and potential treatments for drug abuse begins with preclinical research. Sex differences in responsiveness to drugs in rodents have been reported for over 50 years (Hughes, 2007). Despite this evidence, the vast majority of behavioral pharmacology research continues to use only male subjects and findings are generalized to female populations. Furthermore, the use of only male subjects is not limited to pharmacological and addiction research, (Becker, McClellan, & Reed, 2017). Progress toward the inclusion of females has remained stagnant in all areas of preclinical research for several decades.

There are several reasons why females are omitted as subject in preclinical research (Hughes, 2007). The most frequently cited reason is that the female estrous cycle may confound results by causing variability in experimental data. Another reason is the added cost of using both sexes. Lastly, earlier research failed to yield consistent significant differences between males and females, indicting there was no reason to study both sexes. Thus, preclinical researchers tend to conduct research investigations using only males as subjects to avoid the aforementioned complications (Hughes, 2007).
Previous research from both animal and human studies has indicated that males and females differ in gonadal hormones, brain structures, neurochemistry, pharmacokinetics, pharmacodynamics, and behavioral responses to drugs of abuse (Becker & Hu, 2008; & Hughes, 2007). Hormonal differences between males and females are perhaps a primary reason for sex differences in response to recreational drugs. Several studies indicate that the female rat’s estrous cycle and specifically, ovarian hormones play an important role in sex differences observed in animal models of drug abuse (Becker & Hu, 2008; Hughes, 2007). For example, the estrous cycle has been studied quite extensively in drug-self administration studies using psychostimulants. Results from these studies have suggested that estrogen enhances female rat’s drug-taking behaviors (Becker & Hu, 2008).

Recently, the National Institute of Health (NIH) created a policy mandating the consideration of using female animals as subjects in preclinical research (NIH, 2015). Furthermore, NIH has recognized the importance of the inclusion of females as subjects and soon, failing to take sex into account will make applicants noncompliant when receiving NIH funding. For all applications submitted to the NIH after January 25, 2016, the NIH expects that sex as a biological variable will be “factored into research designs, analyses, and reporting in vertebrate animal and human studies…Strong justification from the scientific literature, preliminary data, or other relevant considerations must be provided for applications proposing to study only one sex” (NOT-OD_15-102, 2015, p.1). As such, the NIH has set forth expectations for future research to consider sex differences when planning experimental designs and data analysis and when reporting results.
LSD Historical Background

Lysergic acid diethylamide (LSD) was first synthesized in 1938 by Dr. Albert Hofmann, a medicinal chemist working for the Sandoz Company in Switzerland (Hintzen & Passie, 2010). Sandoz was interested in alkaloids obtained from ergot, a substance produced by the fungus *Claviceps purpurea*. Hofmann began to combine lysergic acid, the core chemical structure in all ergot alkaloids, with other compounds to create new circulatory and respiratory stimulants. The twenty-fifth compound synthesized in the course of his research was named LSD-25. However, LSD-25 failed to show any significant results and research was discontinued. Five years later, Hofmann elected to re-evaluate LSD-25, considering it might have useful pharmacological properties not observed during initial investigations. While synthesizing a new batch of LSD-25, he accidentally ingested some and experienced bizarre visions for the next few hours. To determine whether the effects he experienced were due to LSD-25, he conducted a self-experiment with 250 micrograms (0.25 milligrams) of LSD three days later (Hintzen & Passie, 2010). Hofmann was excited about his discovery and informed his colleagues at Sandoz that LSD-25 may have some useful implications (Hofmann, 2009).

In 1947, Sandoz marketed LSD under the tradename Delysid (D-Lysergsaurediethylamid) and distributed it in large quantities to scientists in the medical community. It was promoted as an experimental tool that could enhance understanding of mental illnesses at the chemical and behavioral level, such as schizophrenia, psychoses, and neuroses (Hintzen & Passie, 2010). Additionally, LSD was used as a psychotherapy tool to promote psychological insight and for the treatment of alcoholism. During 1950s, interest from other sectors of society developed. The Central Intelligence Agency (CIA)
conducted experiments with LSD as a potential truth serum or mind-control drug in a secret program coded MK-ULTRA. However, in the mid-1960s the program was discontinued because it failed to produce expected results (Hintzen & Passie, 2010).

By the mid-1960s, LSD had moved from the laboratory into the streets and quickly became a popular recreational drug associated with the counterculture movement (Hintzen & Passie, 2010). LSD was further stigmatized by the public media due to adverse . In 1966, LSD was made illegal under provisions of the Federal Drug Abuse Control Amendments and it quickly fell out favor among medical and scientific communities. In 1970, the Drug Enforcement Agency (DEA) under the Controlled Substances Act classified LSD as a Schedule I drug. During the 1970s, as a consequence of the political pressure due to the increasing recreational use, clinical research with LSD in humans virtually stopped. However, recreational use of LSD continued (Hintzen & Passie, 2010) and remains prevalent, particularly among young adults. According to SAMSHA 2014 National Survey on Drug Use and Health (NSDUH), an estimated 287,000 Americans reported current LSD use (defined as use within the last 30 days).

Despite the current status as Schedule I drug, LSD does not meet any of the criteria for a Schedule I drug (i.e., high abuse potential, no accepted medical uses or benefits, there is a lack of accepted safety for its use under medical supervision) for several reasons. First, LSD can cause significant dangers to the user, but not as a result of any pharmacological toxicity like other drugs of abuse (Nichols, 2004). Secondly, LSD does not directly cause overdose deaths, although deaths have occurred in unsupervised settings, mainly due to impaired judgment. LSD has a low risk for acute toxicity and chronic use does not cause organ damage. Lastly, LSD is not addictive and cessation following repeated use does not produce withdrawal symptoms.
Moreover, in animal models of self-administration, LSD is typically not reinforcing to animals. However, LSD can produce tolerance after repeated use, where higher doses are required to obtain the same effects (Nichols, 2004).

**LSD Pharmacology**

LSD is classified as a hallucinogenic drug that alters sensory processing in the brain, and causes perceptual disturbances in visual and auditory modalities, distorted thinking, mood instability, and a loss of contact with reality (Nichols, 2004). The classic hallucinogens are a diverse collection of substances with structural similarities to serotonin or the catecholamines. The classical hallucinogens consist of two broad categories, indolealkylamines (also called indoleamines) and phenylalkylamines, each with subclassifications based on structural differences (Glennon, 1994). The indolealkylamines consist of several subclasses, including tryptamines (e.g., psilocybin, dimethyltryptamine) and ergolines (e.g., LSD). The phenylalkylamines consist of phenylethylamines (e.g., mescaline) and phenylisopropylamines [e.g., 1-(2,5-dimethoxy-4-methyl-phenyl)-2-aminopropane (DOM)]. Despite differences in their chemical structure and potency, the classic hallucinogens tend to produce similar psychological effects through similar pharmacological actions in humans and animals (Nichols, 2004).

LSD can be administered by various routes. Oral administration is most common, through blotter paper, sugar cubes, or by pill (Hintzen & Passie, 2010). In animal research, LSD is typically administered parenterally, either through intraperitoneal (i.p.), intravenous (i.v.), or intramuscular (i.m.) injection. LSD is remarkably potent. In humans, a typical oral dose is between 100-250 micrograms (μg). It is absorbed rapidly from the gastrointestinal tract and distributed throughout the body within 60 minutes. LSD diffuses easily into the brain, however only about 0.01% of the original dose reaches the brain. Following ingestion, the
largest concentration of LSD is in the liver, where the drug is metabolized before it is excreted to 2-oxo-3-hydroxy-LSD. In humans, the biological half-life is estimated to be 2-5 hours and it is eliminated rapidly from the body, with a typical duration of action ranging from 9 to 36 hours. In rodents, LSD’s half-life is reported to be approximately 15 minutes (Hintzen & Passie, 2010).

**Psychological Effects**

In humans, LSD exerts a wide range of psychological effects that include tactile, visual, and auditory alterations in the perception of external stimuli, synesthesia, changes in mood and cognition, illusions, time distortions, and visual hallucinations (Nichols, 2004). LSD’s psychological effects depend on two key factors: the mindset of the user (i.e., personal expectations, and mood) and the ‘setting’ or environment where the user administers LSD. Sometimes unpleasant psychological reactions occur, including panic, confusion, and anxiety. Alterations of consciousness and cognition produced by hallucinogens can also mimic certain psychological disturbances observed in psychotic disorders, such as schizophrenia. Furthermore, hallucinogenic drugs have been reported to induce the onset of psychosis in people predisposed to schizophrenia (Nichols, 2004).

Although the effects of LSD are unpredictable and vary among individuals, higher doses typically produce more intense effects (Hintzen & Passie, 2010). LSD’s psychological effects are typically characterized with four phases. In the first phase, beginning shortly after administration of LSD, and lasting up to two hours, the user observes a release of inner tension. Other characteristics of this phase include laughing or crying, a feeling of euphoria, restlessness, heightened awareness of the environment, and enhanced connection with others. The second phase begins between 30 to 90 minutes after ingestion and is usually marked by perceptual
distortions, such as visual illusions and changes in shapes and colors. The third phase begins three to four hours later and the user often perceives a distorted sense of time, in which time seems to move slowly. Additionally, this phase may produce mood swings, ego disintegration, and a loss of contact with reality. Users often refer to this phase as reaching ‘peak’, in which the maximum effects of LSD are perceived. Finally, four to six hours after administration the effects of LSD begin to lessen and the user begins to return to normal (Hintzen & Passie, 2010).

**Physiological Effects**

LSD produces physiological changes about 30 minutes after drug administration (Hintzen & Passie, 2010). Sympathetic effects include blurred vision, palpitations, increased blood pressure and heart rate, and pupillary dilation. Parasympathetic effects include nausea, loss of appetite, vomiting, headache, dizziness, sweating, and muscle tension. The intensity of autonomic effects is dose-dependent (Hintzen & Passie, 2010).

In rats, LSD produces several distinctive behaviors, including head twitches, ‘wet dog shakes’ involving the whole body, rotational spinning in circles, flat body posture, increased startle response, and a reduction in locomotor and exploratory activity (Krebs-Thomson & Geyer, 1996; & Silverman, 1988).

**Toxicity and Addiction**

LSD is remarkably a nonlethal substance with minimal toxicity. The lethal dose (LD$_{50}$) of LSD varies across species and is primarily related to brain weight rather than body weight (Hintzen & Passie, 2010). LD$_{50}$ in rats is 15.5 mg/kg and in humans, the LD$_{50}$ has been suggested to be around 14,000 micrograms, which is equivalent to about 140 typical LSD doses. There are no reported human deaths directly due to LSD.
overdose. Most LSD-related fatalities reported are a result of adverse interactions with other
drugs, accidental death, or suicide (Hintzen & Passie, 2010).

There are no withdrawal symptoms associated with LSD use, although tolerance to its
hallucinogenic effects develops rapidly after 4 to 7 days of repeated drug use (Hintzen & Passie,
2010). Tolerance to LSD usually lasts for only about three days, after which its effects can be
experienced again with a typical dose level. Cross-tolerance occurs between LSD and other
classic hallucinogens, such as psilocybin (Hintzen & Passie, 2010).

LSD’s Neurochemical Actions

The neurochemical actions of LSD are unique, complex, and not fully understood in
humans or non-humans. In 1954, Wooley and Shaw recognized that LSD and serotonin (5-HT)
were structurally similar. They hypothesized that LSD somehow blocked the actions of
serotonin. This discovery of the structural relationship between LSD and 5-HT catalyzed a
neuroscience revolution because neurochemistry was first linked to behavior and mental illness,
such as schizophrenia instead of environmental factors (Nichols, 2004). Additionally, the
hypothesis emerged that hallucinogens may act through a common serotonergic mechanism.
However, the restrictions placed upon LSD in the 1960s have made it virtuously impossible to
investigate its neurochemical actions in humans. Thus, the majority of studies investigating the
LSD’s neurochemical actions have been conducted using non-humans

Currently, the general hypothesis among researchers is that LSD’s hallucinogenic effects
are likely due to its actions as an agonist on 5-HT2A postsynaptic receptors in the brain
(Halberstadt, 2015; Hintzen & Passie, 2010; & Nichols, 2004). Additionally, LSD has a
complex pharmacological profile that influences the majority of 5-HT receptors, including, 5-
HT₂c, 5-HT₁A, 5-HT₁B, 5-HT₁D, 5-HT₅A, 5-HT₆, and 5-HT₇. LSD also has affinity for dopamine D₁, D₂, D₃, D₄, and alpha₂ receptors (Hintzen & Passie, 2010; Nichols, 2004). Therefore, the complexity of LSD’s mechanism of action has been a significant topic of research. Researchers have sought to explain how a drug can be so potent and have profound effects on consciousness and perception, but have a high affinity for multiple receptors in the brain.

LSD possesses a complex pharmacological profile that includes direct activation of 5-HT₂A receptors and indirect activation of other receptors and their subtypes through second-messenger systems. The 5-HT₂A receptors presumably play an important role in consciousness and perception, as they are localized on cortical pyramidal cells as well as in the thalamus, particularly in the reticular nucleus (Nichols, 2004). The reticular nucleus acts as a filter that controls the intensity and number of signals entering the thalamus through a negative feedback mechanism. The thalamus processes sensory inputs, including visual, auditory, and somatosensory, and receives afferents from the raphe nuclei (RN) and the locus coeruleus (LC). Although the majority of studies suggest that the prefrontal cortex and thalamus are primarily responsible for the actions of LSD, there is also evidence that the LC contributes to LSD’s effects. The LC sends norepinephrine (NE) projections to all parts of the brain (Nichols, 2004).

Studies have shown that serotonin both hyperpolarizes and depolarizes layer V pyramidal neurons by acting on the 5-HT₁A and 5-HT₂A channels respectively (Nichols, 2004). LSD acts as an agonist on 5-HT₁A receptors in the LC, the RN, and frontal cortex by inhibiting firing and 5-HT release. Normal firing of raphe cells in an awake animal causes 5-HT to be released into cortical areas. The administration of classic
hallucinogens suppresses raphe cell firing either directly through activation of serotonin receptors or indirectly by stimulation of inhibitory GABA neurons (Nichols, 2004).

LSD stimulates 5-HT₂₅ receptors on glutamate axon projections from the thalamus. This causes the cortical pyramidal cells to become excited, while at the same time releases glutamate into cortical neuronal fields (Nichols, 2004). Normally, thalamic projections fire in response to sensory information processed by the thalamus. LSD can cause glutamate to be released in the absence of an appropriate stimulus. As a result, LSD enhances sensitivity/excitability of the cortical processing while at the same time causes glutamate to be released from thalamic afferents that normally signal incoming sensory information to be processed (Nichols, 2004). As a result, LSD leads to an overload of sensory processing capacity in the thalamus, causing dramatic changes in consciousness and perception.

**Brain Imaging Studies**

In a recent study, researchers from Imperial College London conducted a series of experiments using three neuroimaging techniques: arterial spin labeling (ASL), blood oxygen level-dependent (BOLD) measures, and magnetoencephalography (MEG), neuroimaging methods were used to investigate the acute effects of LSD in the brains of 20 healthy volunteers (Carhart-Harris, et al., 2016b). Neuroimaging scans were obtained on two separate days; placebo was administered on the first day and 75 µg LSD (i.v.) was administered the next day. Results suggested that under normal conditions, visual information is processed in the visual cortex. However, under the influence of LSD, there was an increased blood flow toward the visual cortex, and many additional brain areas contributed to visual processing. Additionally, normally the brain consists of independent networks that perform separate perceptual functions. However, under the influence of LSD these independent networks became more unified in
processing sensory information. The participants in the study felt a sense of connectedness with their own environment. This was proportional to a decrease in neural firing produced by LSD (Carhart-Harris, et al., 2016b). Thus, marked changes were observed in neuron electrical activity and network communication patterns were altered because of LSD. This study has helped confirm what has been discovered in preclinical studies.

In behavioral pharmacology, several assays are used to catalogue the effects and to study the underlying mechanisms of drug action with LSD and other psychoactive drugs. These assays include locomotor activity, prepulse inhibition, and drug discrimination. The majority of what we know today about LSD mechanism of action is derived from studies that have employed the drug discrimination assay.

**Drug Discrimination**

Drug discrimination (DD) is a popular and reliable assay that has been used since the 1960s to investigate the subjective or interoceptive effects of psychoactive drugs (Glennon & Young, 2011). A psychoactive drug that alters the central nervous system (CNS) may produce a variety of changes in the user’s mood, perception, and/or behavior. When drug effects are paired with a specific behavior, such as pressing a lever, and that behavior is reinforced, the likelihood of making that same response under the influence of those effects is increased. In other words, the effects produced by a drug can function as a discriminative stimulus that alters the behavior of the subject and can set the occasion for which a specific response will be reinforced. Therefore, DD procedures are designed to approximate the subjective effects of psychoactive drugs, in both humans and non-humans, by establishing a drug as a discriminative stimulus. (Glennon & Young, 2011).
The most commonly used procedure is the two-lever drug discrimination paradigm. In this procedure, animals are trained daily during 20 min sessions, in which lever pressing is reinforced by the presentation of food pellets (Glennon & Young, 2011). Discrimination training reinforces responding on one lever following drug administration, and responses on the other lever after vehicle (i.e. saline). Therefore, the training drug serves as a discriminative stimulus or a cue that influences a specific behavioral response. Over time and after many training sessions, a discrimination develops between the administration of the training drug and vehicle (saline) (Glennon & Young, 2011).

After animals are trained to discriminate a specific dose of a training drug from its vehicle, several experiments or tests can be performed. Test sessions are interspersed between training sessions and typically occur two times per week. The first procedure is used to test for stimulus substitution (or stimulus generalization tests). Tests of stimulus substitution are performed to determine whether a novel drug is like the pharmacological effects of the training drug. Typically, when the animal is presented a novel substance, responding 80% or higher on the drug-appropriate lever indicates that the compound ‘substitutes’ and is pharmacologically like the training drug (Glennon & Young, 2011). In other words, the animal generalizes the stimulus effects of the novel compound to those of the training drug. By testing animals at several doses of a novel compound, and recording response selection, a dose-response curve and the degree of drug substitution can be generated.

After animals are reliably trained, two important considerations in substitution tests can be investigated. First, a thorough dose-response investigation of the training drug can be generated (Glennon & Young, 2011). This is calculated by administering lower and higher doses of the training drug. For example, when lower doses of the training drug are administered,
animals typically make fewer responses on the ‘drug-appropriate lever’ and at a very low dose of
the training drug animals will respond on the ‘vehicle-appropriate lever’. Dose-response curves
can be used to estimate the median effective dose (ED$_{50}$) in which 50% of the animals respond
on the drug-appropriate lever. A second important consideration is investigating the duration of
action of the training drug. This is called a time-course curve which can be determined by either
using shorter or longer pre-session injection times with the training drug or test compounds.

Time course of any drug stimulus cue can be characterized by its latency of onset of action, peak
activity, and total duration of pharmacological effects (Glennon & Young, 2011).

Tests of stimulus antagonism can be used to infer the receptor actions of the training
drug. Drugs that are known to block the receptor actions of known neurotransmitters are
administered prior to administration of the training drug (Glennon & Young, 2011). An
antagonist is said to block the stimulus effects of the training drug if the animal responds 80% or
higher on the vehicle-appropriate lever. DD studies have been applied successfully to the study
of LSD, and have been a reliably tool for discovering the mechanisms of action.

There are several advantages to implementing the DD assay. First, the ease of use;
in general, DD studies are relatively easy to conduct and require little specialized skills,
also these procedures are usually noninvasive by avoiding surgical methods (Glennon &
Young, 2011). A second advantage is specificity of action; highly sensitive and exhibits a
unique molecular specificity. Drugs that have common sites of action produce similar
discriminative effects, also the potency of a drug in producing discriminative effects is
proportional to its affinity for the receptor and intrinsic activity. Another advantage is
measurement of in vivo mechanisms of effect; allows investigation of the total effect of
the drug without limiting the study to one or a few targeted structures, or to one or a few
neurotransmitters. DD provides useful information about the total in vivo mechanisms underlying the psychotropic effects of a drug, for example, mechanisms at different levels determine the behavioral effects of drugs, from molecular (site of action) to the cell-physiological (brain circuitry) to the total state effect (anxiety, hallucination, etc.). A fourth advantage is there is independence b/w choice and rates of responding; the discriminative effects of a drug are usually independent from rates of responding such that drugs that depress responding do not normally interfere with DD.

A third advantage is it has a high predictive validity; results obtained from DD animal studies are generally qualitatively and quantitatively consistent with those obtained using analogous procedures in humans, also DD procedures in animals have a high predictive validity regarding the effects of treatments on drug self-administration in animals. Lastly the DD has the ability to qualitatively compare the effects of different drugs, mixtures of drugs, or different doses of the same drug; animals can be trained to discriminate a drug from vehicle, but also to discriminate the effects of 1 dose of a drug from another dose of the same drug, the effects of one drug from the effects of another drug (‘OR-discrimination’), the effects of a mixture of drugs from vehicle (‘AND-discrimination’), or the effects of a mixture of 2 drugs from the effects of each drug separately (‘AND-OR-discrimination’). These types of discrimination allow investigation of a wide variety of scientific questions regarding not only the mechanisms of discriminative effects of specific drugs, but also the nature of the discrimination itself (Glennon & Young, 2011).

The DD assay is not without limitations. A major limitation is the extensive amount of time required to conduct and complete a single study. For example, in most cases it takes approximately three months to train animals to reach training criteria before testing can begin.
Additionally, the time requirement is further prolonged once discriminative training is established because test sessions are only conducted twice per week.

**LSD and Drug Discrimination**

It has long been established that LSD can function as a discriminative stimulus in the rat (Hirshorn & Winter, 1971). The development of the DD assay in animals and the discovery of specific serotonin ligands have facilitated the identification of the 5-HT receptor subtypes that principally mediate the interoceptive effects of LSD. LSD has been studied quite extensively in DD research because its interoceptive cue is reliable, robust, and reasonably selective (Holohean, White, & Appel, 1982). DD research on LSD has facilitated efforts to understanding the mechanisms of action that mediate many of its complex interoceptive effects of it (Nichols, 2004). Over the past 50 years, research studies have demonstrated central role for 5-HT receptors in the interoceptive stimulus effects of LSD (Nichols, 2004). As mentioned above, LSD exerts is hallucinogenic drug cues primarily on the 5-HT$_{2A}$ receptor. However, LSD has been referred to as a complex compound stimulus because unlike the other classic hallucinogens LSD exerts its effects on many other neurotransmitters (Halberstadt & Geyer, 2011).

One of the first studies to demonstrate that LSD could function as a discriminative stimulus was conducted by Hirschhorn and Winter (1971). In this study, rats were trained to discriminate LSD or mescaline from saline using a two-lever DD assay. The results indicated that LSD and mescaline produced similar interoceptive cues in the rat. The authors concluded that LSD and mescaline might have a common mechanism of action due to cross-generalization and cross-tolerance (1971). Subsequent studies demonstrated that serotonergic antagonists, such as methiothepin, methysergide, cyproheptadine, and cinanserin blocked the stimulus effects of LSD (Kuhn, Winter, &
Appel, 1978). These findings indicate that the discriminative stimulus effects of LSD were due to its effects on 5-HT.

Subsequent research by, Glennon, Titeler, and McKenney (1984) investigated substitution tests with 22 hallucinogens in rats trained to discriminate DOM from saline. They concluded that the 5-HT2 receptor was important for mediating the effects of LSD and other hallucinogens due to strong correlation between the affinity of LSD and other hallucinogens for the 5-HT2 receptor and their potency in humans (1984). This study was important because it led to the discovery of 5-HT2 receptor subtypes, such as 5-HT2A and 5-HT2C. The demonstration that hallucinogens can act as agonists at both subtypes raised the question regarding the relative contributions of these 5-HT2 receptor subtypes to the discriminative stimulus of LSD (Nichols, 2004).

A series of studies were conducted by Fiorella, Rabin, Winter (1995b) to investigate the correlation between the effectiveness of ten 5-HT2 antagonists in blocking stimulus control by LSD with their selectivity for the 5-HT2A and 5-HT2C receptor subtypes. The results suggested that the 5-HT2A receptor could only account for 56% of the variance of the LSD-antagonists, and 5-HT2C receptor did not account for the remaining variance mediated by the LSD interoceptive cues. Thus, these results suggested that 5-HT2A receptor is primarily responsible for mediating the discriminative stimulus effects of LSD. However, interactions with other receptor sites may amplify the stimulus effects of LSD (Marona-Lewicka, and Nichols, 1995; Marona-Lewicka, Thisted, & Nichols, 2005; Marona-Lewicka, & Nichols, 2007; Marona-Lewicka, Chemel & Nichols, 2009; and Winter and Rabin, 1988). Moreover, there is considerable evidence that the 5-HT1A receptor subtype as well as dopamine receptors contribute to the discriminative stimulus.

LSD is unique compared to other hallucinogens because it has affinities for other monoamine receptors other than the 5-HT2A that may be responsible for mediating the interoceptive cues (Nichols, 2004). As mentioned above, LSD has substantial affinity for a variety of other receptors, including all 5-HT receptor subtypes, except 5-HT3 and D1, D2, D3, and D4 receptors (Nichols, 2004). Thus, LSD’s relatively indiscriminate receptor-binding profile suggests that it exerts its effects as a compound stimulus contributing to its overall effect (Halberstadt and Geyer, 2011).

Reissig, Eckler, Rabin, and Winter (2005) conducted a study investigating the involvement of the 5-HT1A receptor using the DD procedure. Combination and substitution tests were conducted using the 5-HT1A agonists, 8-OH-DPAT, buspirone, gepirone, ipsapirone and the 5-HT1A antagonist WAY-100,635 to characterize their effects on LSD discrimination. The results indicated that stimulus control by LSD was augmented by all 5-HT1A receptor agonists, while the 5-HT1A antagonist WAY-100,635 abolished this effect. Thus, the 5-HT1A receptor appears to contribute to the discriminative stimulus effects of LSD. However, their precise role is still not well understood (Nichols, 2004).

Previous research has also suggested that LSD has a time-dependent dopaminergic component. LSD’s effects in humans have been reported to consist of two temporal phases: an early “psychedelic phase” followed by a late “paranoid” phase (Marona-Lewicka, Thisted, & Nichols, 2005). In contrast, the effects of other typtamine and phenethylamine hallucinogens have been reported to consist of a single temporal phase. To further explore this phenomenon,
Marona-Lewicka et al. (2005) investigated the two temporal phases in a DD study using rats. Rats were divided into two groups and the first group was trained to discriminate 0.08 mg/kg with a 30-min pretreatment time and a second group was trained to discriminate 0.16 mg/kg with a pretreatment time of 90-min. The results suggested that lengthening of the pretreatment time of LSD to 90-min resulted in the emergence of a dopaminergic interoceptive cue, as evidenced by the substitution of dopamine receptor agonists. For example, the D\textsubscript{2} agonists apomorphine, N-propyldihydrexidine, and quinelorane all fully substituted only in the rats trained to discriminate LSD with a pretreatment time of 90-min. Additionally, in rats trained with a pretreatment time of 30-min, the LSD stimulus cue was blocked by 5-HT\textsubscript{2A} antagonists. In contrast, in rats trained with a 90-min pretreatment, the LSD stimulus was not fully blocked by 5-HT\textsubscript{2A} antagonists. These findings suggest LSD’s discriminative stimulus effects can be characterized by two temporal phases, the first one being mediated predominantly by 5-HT\textsubscript{2A} receptors and the later phase mediated primarily by D\textsubscript{2} receptors.

Marona-Lewicka and Nichols, (2007) further investigated the delayed temporal dopaminergic effects. Similar to the Marona-Lewicka et al. (2005), two groups of rats were administered LSD at 30-min and 90-min pretreatment time. In this study, many agonists and antagonists were investigated. The results suggested that classical hallucinogens, such as psilocybin and mescaline fully substituted in rats trained with a pretreatment time of 30-min, but not in rats trained with a pretreatment time of 90-min. Additionally, dopamine receptor agonists, such as MDMA, cocaine, ABT-724, aripiprazole, dihydrexidine, WAY, 100635, and SKF 38393 fully or partially substituted in rats trained with a 90-min pretreatment time, but not in the rats trained with a 30-min pretreatment time (2007). Thus, these data suggested the complex pharmacological profile of LSD allows for 5-HT and DA receptor activation mediate the
different temporal phases of LSD discrimination in rats. However, little is known whether LSD affects dopamine receptors directly or indirectly through modulatory effects of 5-HT\textsubscript{2A} receptors.

The majority of DD studies investigating the subjective effects of LSD has exclusively used only males as subjects. Thus, there is currently a significant gap in preclinical research concerning sex as a biologically relevant variable in the discriminative stimulus effects of hallucinogens, especially LSD.

**Sex Differences in Drug Discrimination**

The majority of previous research using the DD assay has been conducted using only males as subjects. In a recent review article, Bevins and Charntikov (2015) investigated sex differences in the drug discrimination literature. Only 17 out of thousands of published scholarly research articles were found to include female animals. Of the 17 articles, sex differences were found in only a few of these studies investigating cocaine, morphine, amphetamine, and MDMA subjective effects (Broadbear, Tunstall, and Beringer, 2011; Craft, Kalivas, & Stratmann, 1996; Craft and Stratmann, 1996; Craft, Heideman, & Bartok, 1999; Krivsky, Stoffel, Sumner, Inman, & Craft, 2006). Thus, potential sex differences in these DD studies have been investigated. However, consistent effects have not been replicated, precluding generalization of these findings.

Craft and Stratmann (1996) conducted a study investigating discriminative stimulus effects of cocaine in male and female adult Sprague-Dawley rats. The results indicated that females acquired cocaine discrimination slightly faster than males, however the difference was not statistically significant. Additionally, the effective dose (ED\textsubscript{50}) was the approximately the same between the sexes. Of particular interest, cocaine’s duration of action was significantly shorter in females than in males (Craft and
Stratmann, 1996). These findings suggest that males and females may differ in the effects of cocaine due to hormonal or pharmacokinetic factors.

Another study conducted by Craft, Heideman, and Bartok (1999) investigated sex differences in gonadal hormones on the discriminative stimulus effects of morphine. Adult male and female Sprague-Dawley rats were gonadectomized (GNDZ) or sham-gonadectomized (SHAM), then trained to discriminate morphine. The results suggested that the $ED_{50}$ was significantly lower in intact females than in males. Additionally, sex differences were found in the substitution tests with agonists buprenorphine and nalbuphine. These drugs substituted in nearly all females (GNDZ and SHAM) and in all the SHAM males, but only four out of the seven GNDZ males. Also, most of the opioid agonists used were significantly more potent in decreasing response rates in the male groups than in the female groups. At the end of the study, hormone replacement for the GNDZ females reinstated the estrous cycle, however it did not change the $ED_{50}$ for morphine discrimination (1999). Therefore, these results suggested that few sex differences were due to hormonal differences. Rather, sex differences may be due to differences in reinforcement frequency between saline and drug conditions observed only in the males.

In a more recent study, Broadbear, Tunstall, and Beringer (2010) investigated the role of the hormone oxytocin in the subjective effects of MDMA in male and female Sprague-Dawley rats using a three-lever DD assay. Male and female rats were trained to discriminate MDMA from amphetamine (AMPH) and saline. The results suggested sex differences in the dose-response curves of both MDMA and AMPH, with females being more sensitive to the subjective effects of these drugs. Additionally, males were more sensitive to the rate-suppressing effects of MDMA, AMPH, carbetocin, and atosiban than females (2010). These findings suggest that
females are more sensitive to the psychological effects of MDMA than males and males are more sensitive to the physical effects of MDMA than females. This may be due to pharmacokinetic factors of MDMA.

Relatively few studies have been conducted using both males and females in DD research. The lack of data and insufficient replication of studies investigating sex differences make data interpretation difficult. To date, no known studies investigated sex differences in the discriminative stimulus effects of LSD. However, a few studies have been conducted using other behavioral assays to study sex differences on LSD effects.

**LSD Effects in Females**

A few studies have investigated sex differences in LSD’s behavioral effects using other behavioral assays, such as locomotor and exploratory behavior, prepulse inhibition, and place conditioning (Meehan and Schechter, 1998; Palenicek, Hlinak, Bubenikova-Valesova, Novak, and Horacek, 2010). For example, Meehan and Schechter (1998) established that sex differences exist in LSD-induced conditioned place preference (CPP) in Fawn Hooded (FH) rats. FH rats were selected for that study because they have a genetic deficiency in serotonergic activities. Results suggested that LSD produced place preference only in the male FH rats. The authors suggested sex differences may be due to serotonergic deficits in FH rats or due to the estrous cycle decreasing metabolism of serotonin in the rat central nervous system (Meehan and Schechter, 1998).

Another study conducted by Palenicek et al. (2010) examined sex differences in the behavioral effects of LSD on exploratory behaviors and an acoustic startle reaction in rats. The main sex differences observed were in locomotor and exploratory behavior. Females were less sensitive to the hypolocomotor effects of LSD and displayed a greater
increase in exploratory behavior than males. Additionally, females had lower sensitivity to the disruptive effects of LSD on an acoustic startle reaction compared to males (Palenicek et al., 2010).

The need for female inclusion in preclinical research is readily apparent. Failure to include females is a major limitation in behavioral pharmacology research. Investigating potential sex differences in animals may translate to clinical research. Currently, little is known about sex differences with regards to the subjective effects of drugs, especially LSD. Uncovering evidence may aid in our understanding of the mechanisms of action, subjective, and behavioral effects of LSD. Previous research using behavioral assays has suggested that sex differences exist in some of the behavioral effects of LSD. The primary aim of the present study was to explore possible sex differences in the discriminative stimulus effects of LSD, and in relative contribution of serotonergic and dopaminergic actions to these effects. Drugs with differential actions on 5-HT and DA receptors were evaluated in male and female Sprague-Dawley rats trained to discriminate LSD.
CHAPTER II
METHODS

Subjects

Eight adult males (280-300g) and eight adult females (160-190g) Sprague-Dawley rats (Charles River Laboratories Inc., Kingston, NY, USA) were housed individually in polycarbonate cages lined with corn cob bedding (Harlan Teklad, Conrad, Iowa). Males and females were housed in separate colony rooms in the animal facilities maintained at constant temperature (20±2 ºC) and humidity (50±5 %) under a 12:12 light/dark cycle with lights on from 7:00a.m. to 7:00 p.m. Water was provided ad libitum in the home cages. Commercial rodent diet (Purina® 5001, Richmond, Indiana) was restricted to daily feeding to maintain animals at 80-90% of free-feeding weights. All procedures were reviewed and approved by the Western Michigan University Institutional Animal Care and Use Committee and were in accordance with the guidelines of the Guide for the Care and Use of Laboratory Animals (National Research Council of the National Academics 2011) and EU Directive 2010/63/EU.

Apparatus

Training and testing were conducted in 16 sound-attenuated operant conditioning chambers (ENV-001; MED Associates Inc., Georgia, VT, USA). Males and females were tested in separate rooms and at different times. Chambers were equipped with two retractable levers and a food pellet dispenser located on the front panel, a house light (28V), and fan. Reinforcers for lever pressing consisted of 45-mg Dustless Precision Pellets® (Product# F0021, BioServ, Flemington, NJ). Experimental events were programmed and controlled using Med-PC software (version IV; MED Associates Inc., St. Albans, VT, USA).
Drugs

Lysergic acid diethylamide (LSD), 2,5-Dimethoxy-4-methylamphetamine (DOM), mescaline, (±)3,4-methylenedioxymethamphetamine (±)MDMA, (+)3,4-methylenedioxymethamphetamine (+)MDMA, (-)3,4-Methylenedioxymethamphetamine (-)MDMA, methylenedioxypyrovalerone (MDPV), mephedrone (4-MMC), (+)3,4-methylenedioxymethylamphetamine (+)-MDA), (-)3,4-Methylenedioxymethylamphetamine (-)-MDA), psilocybin, cocaine, d-amphetamine (AMPH), (see Table 1) were generously provided by the National Institute on Drug Abuse Drug Control Supply Program (Bethesda, MD). All substitution drugs were dissolved in bacteriostatic 0.9% sodium chloride and administered by intraperitoneal (i.p) injection with a 15-min pre-session injection interval in a 1 ml/kg injection volume.

Antagonist drugs (see Table 2) WAY-100,635-HCL, MDL-100,907, haloperidol, SCH-23390-HCL, and pirenperone. SCH-23390-HCL, and haloperidol were provided by Sigma Aldrich Corporation (St. Louis, Missouri), WAY-100,635-HCL were provided by Santa Cruz Biotechnology, Inc. (Dallas, Texas), and MDL-100,907 and pirenperone were provided by NIMH Chemical Synthesis and Drug Supply Program (Research Triangle Park, North Carolina). SCH-23390-HCL and WAY-100,635-HCL were dissolved in 0.9% saline and haloperidol was dissolved in 0.1% HCL, and MDL-100,907, and pirenperone were dissolved in a few drops of acetic acid diluted in H2O. All antagonist drugs were administered i.p. injection with a 60-min pre-session injection interval (except pirenperone 30-min pre-session) in a 1 ml/kg injection. All doses were calculated based on the weights of the salts.

Operant Training Procedures
All chambers and levers were wiped clean with 35% isopropyl alcohol after every experimental session to attenuate olfactory cues remaining on the levers and in the testing environment. To control for olfactory cues between males and females, as mentioned above males and females were housed in different colony rooms, run at separate times, in separate chamber rooms, and in separate chambers. It is important to control for olfactory cues between the sexes because pheromone scents can confound experimental data by interrupting physiologic and behavioral responses (Bind, Minney, Rosenfeld, & Hallock, 2013).

**Preliminary training.** Subjects were acclimated to the operant chambers for two 60-min sessions, one per day for two consecutive days. During these two sessions, no levers were extended and food pellets were delivered under a fixed-time 60 s (FT60) schedule to familiarize the animals with the location and sound of the food hopper. The criterion for proceeding to lever press training consisted of each rat consuming all pellets from the magazine by the end of the 60-min session. All subjects proceeded to lever press training after two sessions.

**Errorless training.** Errorless training sessions lasted 20 min per day and were conducted 6 days per week. Animals were initially trained to lever press either the left or right lever and reinforcement was delivered under a fixed-ratio (FR) schedule that was gradually incremented from FR 1 to FR 20 over the course the training sessions. Once subjects were reliably lever pressing on the FR 20 schedule, errorless training sessions commenced with either the left lever or right lever extended. During this phase, subjects received i.p. injections of either 0.08 mg/kg LSD or saline 15 min prior to the beginning of each session. Half the animals in each training group were reinforced for responses on the right lever following drug injections (D) and for responses on the left lever following saline vehicle injections (V). Conditions were reversed for the remaining animals in each
group. Once subjects were responding reliably on an FR 20 schedule on both the drug-paired and vehicle-paired levers, discrimination training commenced.

**Discrimination training.** Both left and right levers were present during discrimination training sessions. These sessions were 20 min in duration and were conducted only once per day, 6 days a week. Similar to the preliminary training sessions, responding was initially reinforced under a FR 1 schedule that was progressively incremented to a FR 20 schedule under drug and vehicle conditions. Once animals were reliably responding under the FR 20 schedule under both drug and vehicle conditions, this schedule remained in effect for the remainder of the training sessions. Drug and vehicle training sessions were alternated with sessions under the same stimulus conditions occurring no more than twice consecutively. The performance criteria for stimulus control was a minimum of eight out of ten consecutive discrimination trials with an 80% or better correct lever response prior to delivery of the first reinforcer and for the total session.

**Stimulus substitution testing.** Stimulus substitution tests commenced when each subject met the criteria for discrimination training. All subjects completed a minimum of one drug and one vehicle training session between substitution test sessions and were required to meet the 80% appropriate responding prior to the first FR and throughout the duration of each session on the most recent drug and vehicle training sessions prior to each test session. All compounds were administered via i.p. injection 15 min prior to commencing test sessions. Test sessions were conducted under extinction and ended immediately following the completion of 20 consecutive responses on either lever or until 20-min elapsed, which ever occurred first. Every subject was tested on the same dose on test days. Substitution tests occurred no more than two times per week with the test compounds presented in Table 1.

**Table 1**

*Compounds for Substitution Tests*
<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSD</td>
<td>0.01, 0.02, 0.04, 0.08</td>
</tr>
<tr>
<td>DOM</td>
<td>0.125, 0.25, 0.5, 1</td>
</tr>
<tr>
<td>(±) MDMA</td>
<td>0.75, 1.5, 3</td>
</tr>
<tr>
<td>Mescaline</td>
<td>2.5, 5, 10</td>
</tr>
<tr>
<td>(+)-MDMA</td>
<td>0.75, 1.5, 3</td>
</tr>
<tr>
<td>(-)-MDMA</td>
<td>0.75, 1.5, 3, 4.5</td>
</tr>
<tr>
<td>MDPV</td>
<td>0.1, 0.3, 1, 3</td>
</tr>
<tr>
<td>4-MMC</td>
<td>0.3, 1, 3</td>
</tr>
<tr>
<td>(+)-MDA</td>
<td>0.75, 1.5, 3</td>
</tr>
<tr>
<td>(-)-MDA</td>
<td>0.75, 1.5, 3</td>
</tr>
<tr>
<td>Psilocybin</td>
<td>0.25, 0.5, 1.0</td>
</tr>
<tr>
<td>Cocaine</td>
<td>1.0, 3.0, 10</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>0.1, 0.3, 1</td>
</tr>
</tbody>
</table>

*Drugs were tested in the order and dose depicted above*

**Antagonism testing.** Antagonist testing began after all substitution drugs have been tested. All subjects completed a minimum of one drug and one vehicle training session between antagonist test sessions and were required to meet the 80% appropriate responding prior to the first FR and throughout the duration of each session on the most recent drug and vehicle training sessions prior to each test session. All compounds were administered via i.p. injection 60-min pre-session, except for pirenperone, which was 30-min prior to commencing test sessions. Test sessions were conducted under extinction and ended immediately following the completion of 20 consecutive responses on either lever or until 20 minutes elapsed, which ever occurred first. Every subject was tested on the same dose on test days. Antagonist tests occurred no more than two times per week with the test compounds presented in Table 2.

**Table 2**
*Compounds for Antagonist Tests*

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WAY-100,635</td>
<td>0.4, 0.8, 1.6</td>
</tr>
</tbody>
</table>
MDL-100,907 | 0.1, 0.05, 0.025  
Haloperidol  | 0.5, 0.25, 0.125  
SCH-23390   | 0.3, 0.1, 0.03, 0.01  
Pirenpirone  | 0.64, 0.32, 1.28  

*Drugs were tested in the order and dose depicted above

**Data Analysis**

Acquisition of drug stimulus control was determined by the number of discrimination training sessions required to reach criteria in each sex. The mean (±SEM) number of sessions to criterion was calculated for each training group and statistically analyzed with a t-test. Dose-response curves were graphed for each training drug and test compound, with the mean (±SEM) percentage of drug-appropriate lever responses as well as the mean (±SEM) response rate (lever presses per second) plotted as a function of dose.

Dose response curves and response rates were statistically analyzed using General Linear Model (GLM) with training drug as a between-subject comparison and test dose as a within-subject comparison. For drugs that produced full substitution (80% or higher drug-lever responding at any dose), a nonlinear regression was conducted on the dose-response curve to estimate effective dose 50 (ED50) values. Statistical analyses were conducted, and graphs were created using GraphPad Prism (version 6.0) (La Jolla, CA, USA) and Minitab (version 17) software (State College, PA, USA).
CHAPTER III

RESULTS

Discrimination Acquisition

All subjects acquired the LSD – saline discrimination within 40 sessions. The acquisition of 0.08 mg/kg LSD vs. saline discrimination is illustrated in Figure 1A. The mean ± S.E.M. number of discrimination training sessions to meet testing criteria was 25.50 sessions (SD = 10.9) for males and 25.75 (SD = 7.80) for females. Overall, there was no statistically significant difference in discrimination acquisition or on number of responses between males and females. However, there was a statistical significant effect of sex on total response rate between males and females [F[1,14] = 13.15, p ≤ 0.01], with males having higher total response rate (see figure 1B).

Figure 1. Learning curves of male and female rats trained to discriminate 0.08 mg/kg LSD from saline vehicle. Filled circles (●) represent the data obtained from males and filled squares (□) represent data from females. Each data point represents the mean (± SE) for (A) percentage of LSD-lever selection, solid lines represent 0.08 mg/kg LSD and dashed lines represent saline and (B) Total number of response per second.
LSD Dose-Response Curves

Figure 2A-B illustrates the dose-response curves following administration of various doses of LSD (0.01- 0.08 mg/kg) for both males and females. LSD produced dose-dependent increases in LSD-appropriate responding in both sexes (Figure 2A-B top panel). Figure 2A illustrates the LSD dose-response curve prior to the administration of any other test compounds (~12 weeks). LSD dose-dependently increased LSD-lever selection up to the 0.08 mg/kg training dose, which was the only dose to fully-substitution in males and females. The ED$_{50}$ value for LSD discrimination was slightly higher in the females compared to males (males ED$_{50}$ = 0.015; 95% Confidence Interval [CI] = 0.01 - 0.03 mg/kg and (females ED$_{50}$ = 0.023; 95% CI = 0.02 – 0.03 mg/kg), respectively. The slopes of the dose-response curves did not differ significantly between males and females. Response rates after saline administration and LSD administration also were very similar in males and females (Figure 1A bottom panel). There were no statistical significant effect on response rate.

After the completion of all substitution tests (~ 41 weeks), the LSD dose-response curve was generated again (Figure 2B). LSD dose-dependently increased LSD-lever selection up to the 0.08 mg/kg training dose. LSD 0.04 mg/kg and 0.08 mg/kg fully-substituted in males and females (males ED$_{50}$ = 0.024 mg/kg; 95% CI = 0.00 – 1.55 mg/kg) and (females ED$_{50}$ = 0.02; 95% CI = -1.82 – 1.61 mg/kg). The ED$_{50}$ value was slightly higher in the males compared to the females; however, the slopes of the dose-response curves did not differ significantly between males and females. Visual inspection of the curve reveals that the females have higher LSD-lever responding at all test doses. Response rate was not significantly different between sexes or doses.
Figure 2: LSD dose-response curves of male and female rats trained to discriminate 0.08 mg/kg LSD from saline vehicle. Filled circles (●) represent the data obtained from males and filled squares (□) represent data from females. Each data point represents the mean (± SE) for (A) LSD dose-response curve approx. 12 weeks (B) LSD dose-response curve approx. 41 weeks.

LSD Time-Course Curve

Results of the time course tests with 0.08 mg/kg LSD in male and female SD rats are illustrated in Figure 3. The results indicated the onset to the effect (≥ 20% drug-appropriate responding), peak activity and complete substitution (≥ 80% drug-appropriate responding) occurred by 15 and 30 min post-injection in both males (99%) and females.
(99%). A time-dependent decline in LSD-lever responding was evident in both males and females, with partial substitution observed at 60, 90, 120 min. There was no overall sex difference in percentage LSD-appropriate responding during time course tests. However, visual inspection of figure 3 (top panel) displays higher partial substitution in males compared to females at 90 and 120 min post-injection. Statistical analysis failed to detect a significant main effect of time or sex on response rate.

**LSD Time Course**

*Figure 3*: Time course for the discriminative stimulus effects of LSD (0.08 mg/kg) administered at 15, 30, 60, 90, and 120 min pre-injection. Percentage LSD-appropriate responding (top panel) and response rate (bottom panel) in males (●) and females (□). Data points represent group means (± S.E.M.) at each time point.

**Substitution Tests**

The results obtained from General Linear Model (GLM) analyses of the percentage LSD-lever selection and response rate for each test compound assessed for substitution is depicted in Table 3.
Table 3: Statistical Analysis of Substitution Tests

<table>
<thead>
<tr>
<th>Test Drug</th>
<th>df (1)</th>
<th>F (%D)</th>
<th>df (2)</th>
<th>F (Rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DOM</strong></td>
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<td>DOSE =</td>
<td>[3,56]</td>
<td>DOSE =</td>
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<tr>
<td></td>
<td>[1,52]</td>
<td>9.11a</td>
<td>[1,56]</td>
<td>3.96b</td>
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<tr>
<td></td>
<td>[3,52]</td>
<td>SEX =</td>
<td>[3,56]</td>
<td>SEX =</td>
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<tr>
<td></td>
<td></td>
<td>0.57</td>
<td>[1,56]</td>
<td>0.251</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOSE*SEX =</td>
<td>[3,56]</td>
<td>DOSE*SEX =</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.32</td>
<td></td>
<td>1.49</td>
</tr>
<tr>
<td><strong>MESCALINE</strong></td>
<td>[2,42]</td>
<td>DOSE =</td>
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<td>DOSE =</td>
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<td></td>
<td>[1,42]</td>
<td>22.82a</td>
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<td></td>
<td></td>
<td>15.27a</td>
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<td></td>
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<td></td>
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<td>DOSE*SEX =</td>
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<td></td>
<td></td>
<td>9.83a</td>
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<td>2.56</td>
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<td><strong>PSILOCYBIN</strong></td>
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<td></td>
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*Note. Each dose response curve was analyzed using GLM. DOSE=treatment; Sex=difference between males (n=8) and females (n=8); DOSE*SEX= interaction  *p< 0.001;  b p< 0.05.*
Hallucinogens

Substitution test results with three classical hallucinogens are presented in Figure 4A-C. Percentage LSD-lever selection and response rates following substitution tests with DOM (0.125 - 1 mg/kg) are illustrated in figure 4A. DOM completely substituted in the males at the 0.5 mg/kg (86%) and partially substituted at the 1 mg/kg (72%) (males $ED_{50} = 0.26$ mg/kg; CI = 0.13 – 0.5 mg/kg). DOM completely substituted in females at the 0.5 mg/kg (97%) and 1 mg/kg (88%) (females $ED_{50} = 0.22$ mg/kg; CI = 0.13 – 0.37 mg/kg) and partially substituted at 0.125 mg/kg DOM. Statistical analysis revealed a significant main effect of test dose on percentage LSD-lever responding, but no significant effect of sex. Tukey’s post hoc test were significant between 0.125 mg/kg and both the 0.5 mg/kg ($p < 0.001$) and 1 mg/kg ($p < 0.01$), between 0.05 mg/kg and the 0.25 mg/kg ($p < 0.0001$), and between the 0.25 mg/kg and the 1 mg/kg ($p < 0.001$) test doses. Statistical analysis of response rate revealed a significant main effect of dose, but not of sex. Tukey’s post hoc test was significant between 0.125 mg/kg and both the both the 0.5 mg/kg ($p < 0.03$) and 1 mg/kg ($p < 0.001$) test doses.

The percentage LSD-lever selection and response rates with mescaline (2.5 - 10 mg/kg) are illustrated in Figure 4B. Mescaline dose-dependently increased LSD-lever selection up to the 10 mg/kg dose in the females. Mescaline fully substituted in the females after the 10 mg/kg (90 %) test dose ($ED_{50} = 5.505$ mg/kg; CI = 0.5998 – 0.8882 mg/kg) and partially substituted after the 5 mg/kg (76%) test dose. In the males, 10 mg/kg mescaline partially substituted for LSD (60 %) and the 5 mg/kg test dose engendered vehicle-appropriate responding. Statistical analysis revealed a significant main effect of dose, sex, and dose x sex interaction on percentage LSD-lever selection. Tukey’s post hoc test was significant between males and females at the 5 mg/kg and 10 mg/kg dose ($p < 0.0001$) with higher percentage-LSD lever selection in females. Tukey’s
post hoc test were significant between the 2.5 mg/kg and both 5 mg/kg (p < 0.01) and 10
mg/kg (p < 0.0001) and between the 5 mg/kg and 10 mg/kg (p < 0.005) test doses.
Response rates following mescaline tests are illustrated in Figure 4B (bottom panel).
Statistical analysis revealed a statistically significant main effect of dose and sex (see
table 3). Tukey’s post hoc test revealed significant effect of sex with higher response rate
in males than females at both the 5 mg/kg (p ≤ 0.05) and 10 mg/kg (p ≤ 0.01) test doses.
Tukey’s post hoc test revealed a statistically significant difference between response rates
following 2.5 mg/kg and 10 mg/kg test dose (p < 0.05) and between 5 mg/kg and 10
mg/kg (p < 0.001).

The percentage LSD-lever selection and response rates with psilocybin (0.25 - 1
mg/kg) are illustrated in Figure 4C. Psilocybin dose-dependently increased LSD-lever
selection up to the 1 mg/kg dose in both sexes. The highest dose of psilocybin (1 mg/kg)
substituted completely in the females (84%) (ED_{50} = 0.41 mg/kg; CI = (-0.52 – 0.24) and
partially substituted in the males (78%). Statistical analysis revealed a significant main
effect of test dose on percentage of LSD-lever responding, but no significant main effect
of sex. Tukey post hoc test revealed a significant difference between the 0.25 mg/kg and
both the 0.5 mg/kg (p < 0.005) and 1 mg/kg (p < 0.001) test doses. There was no
statistically significant effect of dose or sex on response rate. However, visual inspection
of figure 4C (bottom panel) reveals the response rate was higher in the males than the
females at 0.25 mg/kg and 0.5 mg/kg test doses, respectively.
Figure 4: Results of substitution tests with three hallucinogens (A) DOM, (B) mescaline, and (C) psilocybin in males (●) and females (□) trained to discriminate 0.08 mg/kg LSD from saline. Mean (± S.E.M.) percentage LSD-lever selection (top panel) and response rate (bottom panel).

MDMA and MDMA Isomers

The results of substitution tests with (±)MDMA, (+)-MDMA, and (-)-MDMA are shown in Figure 5A-C. The percentage LSD-lever selection (top panel) and response rates (bottom panel) with (±)MDMA (0.75-3 mg/kg) are illustrated in figure 5A. The highest dose of (±)MDMA (3 mg/kg) substituted partially for LSD in only the males (69%). There was no statistically significant main effect of dose or sex. Statistical analysis revealed a significant main
effect of dose and of sex on MDMA response rate (see Table 5). Tukey’s post hoc test revealed a significant effect on response rate at 0.75 mg/kg (p < 0.05) and 1.5 mg/kg (p < 0.005) test dose in the males compared to females. Tukey’s post hoc test was significant (p between the 3 mg/kg and both the 0.75 mg/kg (p < 0.005) and 1.5 mg/kg (p < 0.01) test doses.

Figure 5B illustrates the percentage LSD-lever selection (top panel) and response rates (bottom panel) following (+)-MDMA (0.75 – 3 mg/kg). None of the (+)-MDMA test doses substituted in males or females. Statistical analysis revealed a significant main effect of sex on percentage LSD-lever responding. Tukey’s post hoc test revealed significant effect of sex, males having higher LSD-lever responding at 3 mg/kg (p < 0.05) test dose compared to females. Statistical analysis failed to reveal a significant effect on response rate of dose or sex.

The percentage LSD-lever selection (top panel) and response rates (bottom panel) with (-)-MDMA (0.75 – 3 mg/kg) are illustrated in Figure 5C. The highest dose of (-)-MDMA (3 mg/kg) partially substituted for LSD in the females (65%). No test doses of (-)-MDMA substituted for LSD in the males. Statistical analysis revealed a significant effect of dose on percentage of LSD-lever responding, but no significant effect of sex. Tukey’s post hoc test revealed a significant difference between the 3 mg/kg and both the 0.75 mg/kg (p < 0.001) and 1.5 mg/kg (p < 0.01) test doses. Statistical analysis revealed a significant effect of dose on response rate, but not sex. Tukey’s post hoc test revealed a significant difference between 0.75 mg/kg and the 3 mg/kg (p < 0.01) test dose.
**MDMA & MDMA ISOMERS**

![Graphs showing the effects of MDMA and its isomers on LSD lever selection and response rates.]

*Figure 5:* Results of substitution tests with (A) (+)MDMA (0.75-3 mg/kg), (B) (+)-MDMA (0.75-3 mg/kg), and (C) (-)-MDMA (0.75-4.5 mg/kg) in males (●) and females (□) trained to discriminate 0.08 mg/kg LSD from saline. Mean (± S.E.M.) percentage LSD-lever selection (top panel) and response rate (bottom panel).

**MDA Isomers**

The percentage LSD-lever selection (top panel) and response rates (bottom panel) with (+)-MDA (0.75 - 3.0 mg/kg) are illustrated in Figure 6A. (+)-MDA 1.5 mg/kg (64%) and 3 mg/kg (62%) partially substituted in the males. None of the doses tested substituted for LSD in the females. Statistical analysis revealed no significant effect of (+)-MDA dose or sex on percentage of LSD-lever responding (see Table 5). Statistical analysis of response rate revealed...
a significant effect of (+)-MDA dose, but not of sex. Tukey’s post hoc test revealed a significant difference between the 0.75 mg/kg and the 3 mg/kg (p < 0.004) test doses.

The percentage LSD-lever selection (top panel) and response rates (bottom panel) following (-)-MDA (0.75 - 3.0 mg/kg) are illustrated in Figure 6B. (-)-MDA 1.5 mg/kg (68%) and 3 mg/kg (73%) partially substituted in the females. None of (-)-MDA test doses substituted in the males. Statistical analysis revealed no significant effect of (-)-MDA dose or of sex. Statistical analysis of response rates revealed a significant effect of (-)-MDA dose, but not sex. Tukey’s post hoc test revealed a significant difference between the 0.75 mg/kg and the 3 mg/kg (p < 0.01) test doses.

**MDA ISOMERS**

*Figure 6:* Results of substitution tests with (A) (+)-MDA (0.75- 3 mg/kg), and (B) (-)-MDA (0.75- 3 mg/kg) in males (●) and females (□) trained to discriminate 0.08 mg/kg LSD from saline. Mean (± S.E.M.) percentage LSD-lever selection (top panel) and response rate (bottom panel).
**Synthetic Cathinones**

The percentage LSD-lever selection (top panel) and response rates (bottom panel) with MDPV (0.1 – 3 mg/kg) are illustrated in Figure 7A. MDPV 3 mg/kg partially substituted in the females (71%). No test doses of MDPV substituted in the males. Statistical analysis failed to reveal a significant effect of MDPV dose or sex on percentage of LSD-lever responding (see Table 5). Statistical analysis on response rate revealed a significant main effect of dose, but not on sex. Tukey’s post hoc test revealed a significant effect between the 0.1 mg/kg and both the 0.3 mg/kg (p < 0.04) and 3 mg/kg (p < 0.01) test doses and between the 1 mg/kg and 3 mg/kg (p < 0.03) test doses.

The percentage LSD-lever selection (top panel) and response rates (bottom panel) following 4-MMC (0.3 – 3 mg/kg) are illustrated in Figure 7B. The highest dose of 4-MMC (3 mg/kg) partially substituted in the females (68%). No test doses of 4-MMC substituted in the males. Statistical analysis revealed a significant effect of sex, but not of dose. Tukey’s post hoc test revealed a significant difference between males and females at 3 mg/kg test dose (p < 0.005). Statistical analysis on response rate failed to reveal a significant effect of dose or sex.

**Stimulants**

The percentage LSD-lever selection (top panel) and response rates (bottom panel) with cocaine (1 – 10 mg/kg) substation tests are illustrated in Figure 8A. No dose of cocaine substituted for LSD in males or females. However, visual comparison indicated that the females exhibited a higher percentage LSD-lever selection at all test doses compare to males. Statistical analysis revealed a significant effect of sex, but not dose (see Table 5). Statistical analysis on response rate revealed a significant effect of sex, but not of dose. Tukey’s post hoc test revealed
a significant difference between males and females following 1 mg/kg (p < 0.05) and 3 mg/kg cocaine (p < 0.05).

The percentage LSD-lever selection (top panel) and response rates (bottom panel) following \textit{d}-amphetamine (AMPH) (0.1 – 1 mg/kg) are illustrated in Figure 8B. One of the male rats died during testing AMPH and its data are not included in the analysis. Statistical analysis revealed no significant effect of AMPH dose or sex on percentage LSD-lever responding. Statistical analysis on response rate revealed no significant main effect of dose or sex.

**Synthetic Cathinones**

\textbf{Figure 7}: Results of substitution tests with (A) MDPV, and (B) 4-MMC in males (●) and females (□) trained to discriminate 0.08 mg/kg LSD from saline. Mean (± S.E.M.) percentage LSD-lever selection (top panel) and response rate (bottom panel).
Figure 8: Results of substitution tests with (A) cocaine, and (B) d-amphetamine in males (●) and females (□) trained to discriminate 0.08 mg/kg LSD from saline. Mean (± S.E.M.) percentage LSD-lever selection (top panel) and response rate (bottom panel).

Antagonists Tests

Serotonin Antagonists

To determine the role of various 5-HT receptors in mediating the discriminative stimulus effects of LSD, rats were pretreated with three 5-HT antagonists prior to the administration of LSD (0.08 mg/kg). One male rat died prior to starting the antagonist tests.
Table 4: Statistical analysis using a GLM on each antagonist administered prior to the administration of 0.08 mg/kg LSD.

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Note. Each dose response curve was analyzed using a two-way repeated measures ANOVA

DOSE=treatment; Sex=difference between males (n=7) and females (n=8); DOSE*SEX=interaction; a p< 0.001; b p< 0.05.

5-HT Antagonists

Figure 9A shows the percentage LSD-lever selection (top panel) and response rates (bottom panel) with WAY 100,635 (0.4 – 0.16) administered in combination with LSD 0.08 mg/kg. None of the test doses of WAY 100,635 completely blocked the LSD stimulus cue in males or females. However, 0.4 mg/kg and 0.8 mg/kg WAY 100,635 partially blocked the LSD stimulus cue in only males. Statistical analysis revealed no significant effect of dose or sex (see Table 4). Statistical analysis on response rate revealed a significant effect of sex, but not dose. Tukey’s post hoc tests revealed a
significant effect of sex (p < 0.05), with females having a higher rate of response at all of WAY 100,635 tests doses.

The percentage LSD-lever selection (top panel) and response rates (bottom panel) following MDL 100,907 (0.025 – 0.1 mg/kg) administered in combination with LSD 0.08 mg/kg are displayed in Figure 9B. MDL 100,907 completely blocked LSD stimulus cue at all doses tested in males. Partial antagonism of the LSD cue at 0.05 mg/kg occurred in the females. Statistical analysis revealed a significant effect of MDL 100,907 dose on percentage of LSD-lever responses, but not sex. Tukey’s post hoc test was significant between the vehicle control and 0.25 mg/kg (p < 0.002), 0.05 mg/kg (0.001), and 0.1 mg/kg (p < 0.005) test doses. Statistical analysis on response rate revealed a significant effect of dose and sex. Tukey’s post hoc tests revealed a significant effect of MDL100, 907 dose between the control dose and both 0.025 mg/kg (p < 0.001) and 0.05 mg/kg (p < 0.0001) and between 0.1 mg/kg and both 0.025 mg/kg (p < 0.001) and 0.05 mg/kg (p < 0.002). Tukey’s post hoc tests revealed a significant effect of sex (p < 0.05), males having higher response rate at all of the test doses compared to females.

The percentage LSD-lever selection (top panel) and response rates (bottom panel) with pirenperone (0.32 – 1.28 mg/kg) administered in combination with LSD 0.08 mg/kg are displayed in Figure 9C. Pirenperone completely attenuated the LSD stimulus cue at all of the doses tested in the males. The intermediate dose of pirenperone (0.64 mg/kg) partially blocked the LSD stimulus cue and the lowest (0.32 mg/kg) and highest (1.28 mg/kg) completely antagonized the LSD cue in females. Statistical analysis of percentage LSD-lever selection and on response rate failed to reveal a statistically significant effect of pirenperone dose or sex.
Figure 9: Results of serotonin antagonist’s tests with (A) WAY 100,635, (B) MDL 100,907, and (C) pirenperone in males (●) and females (□) trained to discriminate 0.08 mg/kg LSD from saline. Mean (± S.E.M.) percentage LSD-lever selection (top panel) and response rate (bottom panel).

Dopamine Antagonists

Figure 10 A shows the percentage LSD-lever selection (top panel) and response rates (bottom panel) following SCH 23390 (0.010 – 0.3 mg/kg) administered in combination with LSD 0.08 mg/kg. SCH 23390 failed to block the LSD stimulus cue at all tested doses in both males and females. Statistical analysis on percentage LSD-lever responding failed to reveal a significant main effect of SCH 23390 dose or sex (see Table 4). Statistical analysis on response rate revealed a significant effect of dose and sex. Tukey’s post hoc test revealed a significant
effect between SCH 23390 0.01 mg/kg and 0.1 mg/kg (p < 0.02). Tukey’s post hoc test revealed a significant effect of sex (p < 0.05), females having a higher response rate at all test doses compared to males.

The percentage LSD-lever selection (top panel) and response rates (bottom panel) with haloperidol (0.125 – 0.5 mg/kg) administered in combination with LSD 0.08 mg/kg are illustrated in Figure 10B. Haloperidol failed to attenuate the LSD stimulus cue at all of the doses tested in both males and females. Statistical analysis on percentage LSD-lever responding failed to reveal a significant effect of haloperidol of dose or sex. Statistical analysis on response rate revealed a significant main effect on sex, but not on haloperidol dose. Tukey’s post hoc test reveal a significant effect on response rate at 0.125 mg/kg ((p < 0.05), males having higher response rate compared to females.

**Dopamine Antagonists**

*Figure 10*: Results of dopamine antagonist’s test with (A) SCH 23390, and (B) in males (●) and females (□) trained to discriminate 0.08 mg/kg LSD from saline. Mean (± S.E.M.) percentage LSD-lever selection (top panel) and response rate (bottom panel).
CHAPTER IV

DISCUSSION

The purpose of this study was to investigate the discriminative stimulus effects of LSD in adult male and female Sprague-Dawley rats (SD). This is the first known preclinical study to investigate possible sex differences in the pharmacological mechanisms contributing to LSD’s discriminative stimulus effects. The current results obtained are consistent with previous LSD discrimination studies conducted in only male rats (Appel, West, Rolandi, Alici, Pechersky, 1999; & Marona-Lewicka, et al., 2007; Marona-Lewicka, et al., 2009; Glennon & Young, 2011). In the present study, there were several sex differences in LSD discriminative stimulus effects across numerous parameters. For example, sex differences were found in substitution tests with other drugs (see Table 5). However, the average number of sessions to establish the LSD discrimination and the potency of LSD were nearly equivalent in males and females.

The average number of sessions to establish LSD discrimination is in general agreement with previous research in only male rats. For example, previous research has suggested that the mean number of sessions to training criteria using 0.08 mg/kg LSD was approximately 25 to 30 sessions (Appel et al., 1999; & Glennon & Young, 2011). In the present study, males and females did not differ in the acquisition of the LSD – saline discrimination. The number of sessions to meet criteria was approximately 25 sessions for both males and females. Additionally, the ED50 for LSD substitution in the present study is within the range reported previously for males trained to discriminate LSD (~0.037 mg/kg) (Appel et al., 1999; Nichols, 2004).
Table 5: Substitution tests in male and female rats trained to discriminate LSD (0.08 mg/kg) from saline

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Males (n=8)</th>
<th>Females (n=8)</th>
<th>Statistical Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOM</td>
<td>CS- 0.5</td>
<td>CS- 0.5, 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PS- 1</td>
<td>PS- 0.125</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NS- 0.125, 0.25</td>
<td>NS- 0.25</td>
<td></td>
</tr>
<tr>
<td>Mescaline</td>
<td>PS-10</td>
<td>CS- 10, 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NS- 2.5, 5</td>
<td>NS- 2.5</td>
<td></td>
</tr>
<tr>
<td>Psilocybin</td>
<td>PS- 0.5, 1</td>
<td>CS- 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NS- 0.25</td>
<td>PS- 0.5</td>
<td></td>
</tr>
<tr>
<td>(±)MDMA</td>
<td>PS- 0.75, 1.5, 3</td>
<td>NS- 0.75, 1.5, 3</td>
<td>Yes</td>
</tr>
<tr>
<td>(-)MDMA</td>
<td>PS-3</td>
<td>PS- 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NS- 0.75, 1.5</td>
<td>NS- 0.75, 1.5</td>
<td></td>
</tr>
<tr>
<td>(+)-MDA</td>
<td>PS- 1.5, 3</td>
<td>PS- 0.75, 1.5, 3</td>
<td>Yes</td>
</tr>
<tr>
<td>(-)-MDA</td>
<td>PS- 1.5, 3</td>
<td>PS- 1.5, 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NS- 0.75</td>
<td>NS- 0.75</td>
<td></td>
</tr>
<tr>
<td>MDPV</td>
<td>NS- 0.1, 0.3, 1, 3</td>
<td>PS- 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NS- 0.1, 0.3, 1</td>
<td>NS- 0.1, 0.3, 1</td>
<td></td>
</tr>
<tr>
<td>4-MMC</td>
<td>NS- 0.3, 1, 3</td>
<td>PS- 1, 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NS- 0.3</td>
<td>NS- 0.3</td>
<td></td>
</tr>
</tbody>
</table>

Note: CS=complete substitution (≥80%); PS= partial substitution (60-80%); NS= no substitution (≤ 60%). Doses are in mg/kg.

Previous research in only male rats trained to discriminate LSD from saline indicates a time course of LSD discrimination similar to that in the present study (Marona-Lewicka, et al., 2007; Marona-Lewicka, et al., 2009; Nichols, 2004). In the present study, there were no statistically significant differences between males and females regarding the time course of LSD’s discriminative stimulus effects. However, visual inspection of Figure 3A indicates that at 90 min, females showed less LSD-appropriate responding (23%) compared to males (47%).
LSD’s main mechanism of action is thought to be governed by 5-HT$_{2A/C}$ and 5-HT$_{1A}$ receptors. Previous research has suggested that 5-HT$_{2A/C}$ and 5-HT$_{1A}$ receptors are strongly influenced by female hormones, estrogen and progesterone (Cosgrove, Mazure, & Staley, 2007; & Cyr, Landry, & Di, 2000). Thus, female sex hormones may influence the expression and/or sensitivity of 5-HT receptors. Based on previous research we might expect female sex hormones to play an important role in the sensitivity of the serotonergic system to LSD’s interoceptive effects. However, further research is needed to investigate pharmacological differences between males and females.

The current results regarding stimulus substitution with other hallucinogens are consistent with previous research conducted only in male rats. For example, DOM, mescaline, and psilocybin have all been shown to fully substitute for LSD in male rats discriminating a range of doses of LSD (0.01 – 0.08 mg/kg) from saline (Glennon & Young, 2011; Appel et al., 1999; & Nichols, 2004). Previous research has suggested that DOM, mescaline, and psilocybin exert hallucinogen-induced stimulus control through common mechanisms of action with 5-HT receptor sites (Nichols, 2004). In the current study, the main sex difference was that females exhibited higher levels of LSD substitution with DOM, mescaline, and psilocybin than the males (see table 5).

Previous research with male rats discriminating LSD are in general agreement with the current results concerning (±)MDMA, (+)-MDMA, (-)-MDMA, (+)-MDA, and (-)-MDA (Baker, et al., 1995; Baker & Taylor, 1997; & Baker, Virden, Miller, & Sullivan, 1997). Previous research findings indicate the enantiomers of MDA and MDMA differ in the extent to which they produce substitution in rats trained to discriminate $d$-amphetamine (AMPH) or classical hallucinogens, such as LSD. For
example, (+)-MDMA and (+)-MDA substituted for AMPH and (-)-MDMA and (-)-MDA substituted for the hallucinogens DOM and LSD (Callahan & Appel, 1988). Additionally, previous research has suggested that (+)-MDMA and (+)-MDA isomers are more potent dopamine (DA) releasers, are more similar to d-amphetamine (AMPH), and cause more disruption on operant responding than the (-)-MDMA and (-)-MDA isomers (Baker, et al., 1995; Baker & Taylor, 1997; & Baker, et al., 1997). In contrast, (-)-MDMA and (-)-MDA isomers bind to 5-HT$_{2A}$ receptors with a higher affinity, are more similar to classical hallucinogens, and substitute for LSD (Baker & Taylor, 1997).

A somewhat unexpected result in the current study was that (+)-MDMA, (-)-MDMA, (+)-MDA, and (-)-MDA had opposite patterns of partial substitution for LSD between males and females. For example, males exhibited higher percentage of LSD-lever responding after the administration of (+)-MDMA and (+)-MDA than females. On the contrary, females exhibited higher percentage of LSD-lever responding after the administration of (-)-MDMA and (-)-MDA. Previous research has suggested that there is some common mechanism between LSD and MDMA, although LSD acts directly on 5-HT$_2$ receptors while MDMA acts predominantly as a 5-HT releaser. One plausible explanation for the discrepancy between the sexes is female’s sex hormones influencing the expression and activity of 5-HT receptors in regulating the effects of MDMA (Lazenka, Suyama, Bauer, Banks, & Negus, 2017). Another possible explanation for higher LSD-lever responding by males following administration of (+)-MDMA and (+)-MDA is the involvement of dopamine release. Previous research has suggested that dopamine systems are altered by female sex hormones (Becker & Hu, 2009; Lazenka et al., 2017). Thus, females may be more sensitive to serotonergic drugs, such as (-)-MDA and (-)-MDMA and males may be more sensitive to dopaminergic drugs. However, this might not be evident with
phenylalkylamines, such as (±) MDMA and MDA stereoisomers. Further examination of receptor mechanisms may elucidate any sex differences in the mechanisms underlying LSD’s discriminative stimulus effects.

The general failure of stimulants to substitute for LSD in the present study also agrees with previous research conducted only in male rats. For example, cocaine and d-amphetamine (AMPH) did not substitute for LSD in either sex in this study, and previous studies in only male animals trained to discriminate LSD from saline suggested that stimulants, such as AMPH did not substitute for LSD (Glennon & Young, 2011; Marona-Lewicka, et al., 2005; & Marona-Lewicka, et al., 2009). However, even though cocaine or AMPH did not substitute for LSD in either sex, there were statistically significant sex differences in the percentage of LSD-lever responding. As a group, the females exhibited higher LSD-lever responding than males following all doses of cocaine.

The majority of preclinical research investigating sex differences has been conducted with stimulants. For example, previous research has suggested that females showed a greater locomotor activation and higher breaking points in self-administration studies than males after AMPH and cocaine administration (Becker & Hu, 2008; Craft & Stratmann, 1996). Additionally, evidence has suggested that female rats have higher basal levels and higher psychostimulant-induced dopamine release than males (Walker, Francis, Cabassa, & Kuhn, 2001). These studies concluded that sex differences in the activation of dopaminergic systems have been attributed to circulating hormones, as well as dopaminergic functionality (Becker & Hu, 2008; Walker, et al., 2001).

The general pattern of the current results for the antagonist tests in male and female rats agrees with previous LSD discrimination studies conducted in only male rats.
(see table 6). To determine the role of various 5-HT antagonists in mediating the discriminative stimulus effects of LSD, rats were pretreated with increasing doses of WAY 100,635, MDL 100,907, and pirenperone. Additionally, rats were also pretreated with increasing doses of two dopamine (DA) antagonists, haloperidol and SCH 23390. Overall, the main sex difference was the 5-HT antagonists produced greater antagonism of LSD’s discriminative stimulus effects and DA antagonists were more disruptive on response rate in the males than the females.

Table 6: Antagonist tests in male and female rats trained to discriminate LSD (0.08 mg/kg) from saline

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Males (n=6)</th>
<th>Females (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WAY 100,635</td>
<td>PB- 0.04, 0.8</td>
<td>NB- 0.8</td>
</tr>
<tr>
<td></td>
<td>NB- 1.6</td>
<td></td>
</tr>
<tr>
<td>MDL 100,907</td>
<td>CB- 0.025, 0.05, 0.1</td>
<td>CB- 0.025, 0.1</td>
</tr>
<tr>
<td>Pirenperone</td>
<td>CB- 0.32, 0.64, 1.28</td>
<td>CB- 0.32, 1.28</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>PB- 0.5</td>
<td>NB- 0.125, 0.25</td>
</tr>
<tr>
<td>SCH 23390</td>
<td>NB- 0.01, 0.03, 0.1, 0.3</td>
<td>NB- 0.01, 0.03, 0.1, 0.3</td>
</tr>
</tbody>
</table>

*Note: CB=complete blockade (≥80%); PB= partial blockade (40-70%); NB= no blockade (≤ 40%). Doses are in mg/kg*

Previous drug discrimination studies have investigated the possible role for the 5-HT₁A receptor in the stimulus effects of LSD. WAY 100,635 is typically used as a selective 5-HT₁A receptor antagonist. However, evidence from previous research with this antagonist has yielded inconsistent results regarding antagonism of LSD discriminative stimulus effects. For example, WAY 100,635 blocked LSD-lever responding (Reissig, et al., 2005; & Mertel et al., 2007).
Conversely, other researchers have reported that WAY 100,635 had no effect on LSD-lever responding (Gresch, Barrett, Sanders-Bush, & Smith, 2006; & Marona-Lewicka et al., 2009). These authors suggested the effects of WAY 100,635 are mediated by activating dopamine (D4) receptors and not by blocking 5-HT1A receptors. Despite discrepancies, in the present study WAY 100,635 did not completely block LSD-appropriate lever responding in either sex. These findings support previous research that 5-HT1A receptors are not critically involved in the discriminative stimulus effects of LSD.

MDL 100,907, a selective 5-HT2A receptor antagonist, has been shown to completely antagonize the LSD cue in male rats (Gresch et al., 2007; Marona-Lewicka, et al., 2007; and Nichols, 2004). The results of the present study are consistent with previous findings. However, MDL-100,907 produced differential effects between males and females. As evident in figure 10B, the percentage of LSD-lever responding at all MDL 100,907 doses was higher in the females than in the males, and MDL 100,907 completely blocked the LSD cue at all of the doses tested. On the contrary, in the females the highest (0.1 mg/kg) and lowest (0.025 mg/kg) MDL 100,907 doses completely blocked the LSD cue; however, the intermediate dose of MDL 100,907 (0.05 mg/kg) only partially blocked the LSD cue. Thus, the sex difference in MDL 100,907 antagonism effects may reflect a minor sex difference in 5-HT receptor pharmacology. More research is needed to investigate the 5-HT antagonists and female receptor pharmacodynamics.

Numerous studies have demonstrated that the 5-HT2 receptor antagonist pirenperone is highly efficacious at completely blocking the LSD stimulus cue (Colpaert,
Carlos, Niemegeers, & Janssen, 1982; Cunningham & Appel, 1987; and Nichols, 2004). Results of the present study agree with previous research conducted only in male rats. However, the intermediate pirenperone dose (0.64 mg/kg) failed to completely attenuate the LSD stimulus cue in the females. These results may reflect fluctuations in female hormones that impact 5-HT receptor pharmacology.

To summarize, the selective 5-HT$_{2A}$ antagonist, MDL 100,907 and 5-HT$_2$ antagonist pirenperone significantly reduced LSD-lever responding, whereas the selective 5-HT1A antagonist, WAY 100,635 did not significantly alter LSD discriminative stimulus. Dopamine antagonists also failed to block LSD discrimination, although there were apparent sex differences in the rate suppressant effects of these substances. These findings confirm previous research suggesting a major role for the 5-HT$_{2A}$ receptor in mediating LSD’s psychoactive effects.

Limitations of the current study merit some discussion. One methodological constraint in the present study is inherent in the drug discrimination paradigm. Specifically, this study was conducted over an extended period of time (~15 months) and phases of the females’ estrous cycles were neither measured nor controlled. Thus, the specific phases of the estrous cycle for each individual female rat occurred at variable points throughout the duration of this experiment. This methodological constraint may have introduced a source of variability in the data that could have obscured the results of several test drugs. However, Prendergast, Onishi, and Zucker (2014) conducted a meta-analysis examining the variability within male and female mice in 293 publications. Results suggested that compared to males, females were equally or even less variable in all reported physiological and behavioral measures investigated (Prendergast, et al., 2014). Additionally, Becker, McClellan, & Reed (2017) recently reported no significant sex
differences when the estrous cycle in female rats was incorporated as a variable in several behavioral assessments, such as the forced swim test and cocaine self-administration.

On the contrary, Páleníček et al., (2010) investigated the estrous cycle on the behavioral activity of LSD. Females were divided into two groups: EP (estrus and proestrus phases) and MD (metestrus and diestrus phases). Results suggested that LSD mainly inhibited locomotor effects in the MD females, however in ED females, LSD increased locomotor activity during the latter half of the testing period. Furthermore, EP females showed less sensitivity to the disruptive effects of LSD on prepulse inhibition, suggesting EP females were more protected against some of the effects of LSD. The authors concluded that since sex hormones are at their highest level during estrus and proestrus phases, female rats may have an increased number of 5-HT$_{2A}$ receptors (Páleníček et al., 2010). Therefore, based upon this study, it is at least plausible that the interoceptive effects of LSD may vary, contingent upon the estrous cycle phase at the time of administration of LSD and other test compounds.

Another limitation to this study is LSD evokes a compound stimulus (Halberstadt & Geyer, 2011; Winter, 2009). Although the main component of the LSD cue is mediated through 5-HT$_{2A}$ receptors, LSD also binds with high affinity to a variety of other monoamine receptors that may be responsible for minor elements of the LSD stimulus cue. The minor elements of the LSD stimulus cue have been attributed to 5-HT$_{1A}$ and DA D$_2$ and D$_4$ receptors (Appel et al., 1982; Marona-Lewicka and Nichols; 1995; & Nichols, 2004). It is possible that the most salient feature of a compound stimulus varies among individual subjects, such that 5-HT$_{2A}$ receptor-mediated effects of LSD might be the most salient feature in some subjects but not others. Therefore, sex
differences observed in regard to stimulus substitution in the current study may be related to the compound stimulus properties of LSD. The 5-HT$_{2A}$ component of the LSD cue may vary between sexes and among individuals. Future research is required to tease out the differences in receptor pharmacology of LSD in males and females.

Despite the aforementioned limitations of the current study, two conclusions may be made. Male and female adult SD rats differ in the relative contributions of serotonergic and dopaminergic activities to the LSD discriminative stimulus cue, including higher levels of LSD substitution with 5-HT agonists, such as DOM, mescaline, psilocybin, (-)-MDMA, and (-)-MDA in the females than the males and more complete antagonism of LSD discrimination by 5-HT antagonists in males than the females. These findings may suggest that the LSD cue is differentially mediated by 5-HT receptors in males and females. Previous research has suggested that sexual dimorphisms exist in the 5-HT system of the rat brain. For example, striatal 5-HT levels are higher in female SD rats than males (Páleniček et al., 2010). Additionally, female sex hormones, estrogen and progesterone increase the density, expression, and sensitivity of 5-HT receptors (Cosgrove et al., 2007). Therefore, female sex hormones may play an important role in sensitivity to the LSD stimulus cue. Moreover, common mechanisms between drugs, such as LSD and MDMA and MDA isomers that involve both serotonergic and dopaminergic mediation of the discriminative cue may be altered by female sex hormones. Additionally, higher levels of partial substitution by the synthetic cathinones in females and stronger response disruption by dopamine antagonists in males may indicate sex differences in the contribution of dopamine to LSD’s discriminative stimulus effects.

Sex is an important biological variable in both clinical and preclinical research. Preclinical animal models aid in understanding the underlying mechanisms involved with drug
action and the results of these studies lay the groundwork for clinical research. Since, May of 2014 the NIH has been implementing policies regarding the inclusion of female subjects in preclinical work. One of the biggest reasons for this reform in preclinical research is the multitude of published articles that have documented sex differences in gene expression and neurobiology that can affect the pharmacokinetics and pharmacodynamics of drugs. Additionally, the need for preclinical research investigating interactions of female sex hormones on the discriminative stimulus effects of LSD and other drugs.

The results of the current study may be informative for future investigations with clinical populations regarding possible sex differences in the subjective and pharmacodynamic effects of LSD. Clinical experimental research with LSD is currently undergoing a major revival and since 2014, numerous clinical reports investigating the effects of LSD have appeared in the scientific press (Carhart-Harris, Walley, Bolstridge, Feilding, and Nutt, 2014a; Carhart-Harris, et al., 2014b; Carhart-Harris, et al., 2016a; Carhart-Harris, et al., 2016b; Gasser, et al., 2014; Schmid et al., 2014; Dolder, Schmid, Haschke, Rentsch, & Liechti, 2015; Kaelen, et al., 2015; & Liechti, Dolder, & Schmid, 2017). Evidence from these studies has suggested that LSD has promising therapeutic effects in treating various psychiatric disorders, such as anxiety, depression, and treatment of alcoholism (Gasser, et al., 2014; & Dolder, et al., 2015). For example, Gasser et al. (2014) suggested that LSD-assisted psychotherapy significant reduced anxiety in participants diagnosis with a life-threatening disease for up to 12 months. LSD has also been investigated in the treating of alcohol and opiate addiction. For example, Krebs and Johnansen (2012) conducted a meta-analysis study investigating LSD’s effects
in the treatment of alcohol. Results suggested that participants that received LSD had significant decreases in alcohol misuse compared to the control group. Further examination of sex differences in humans is warranted.

Future research using animal models of drug discrimination that examine the factors discussed above may aid in establishing a better understanding regarding any sex differences in neural mechanisms responsible for LSD’s interoceptive stimulus effects. Although the recent trend in drug abuse research has been focusing on both male and females, it is apparent that more research is necessary to clearly define the factors that contribute to sex differences in the interoceptive stimulus effects of LSD and other illicit drugs.
REFERENCES


Páleníček, Hliňák, Bubeniková-Valešová, Novák, & Horáček. (2010). Sex differences in the effects of N,N-diethyllysergamide (LSD) on behavioural activity and prepulse

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APPENDIX

IACUC Approval Form
Date: February 24, 2016

To: Lisa Baker, Principal Investigator

From: Kathryn Eckler, DVM, Vice Chair

Re: IACUC Protocol Number 16-02-05

Your protocol entitled “Drug Discrimination Studies of Psychoactive Drugs 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: February 23, 2017