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## Assessment N>e Discriminative Stimulus Effects of (±)-3,4-Methylenedioxymethamphetamine (MDMA, "ecstasy") and (+Lysergic Acid Diethylamide (LSD) in a Three-Lever Drug Discrimination Procedure

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ASSESSMENT OF THE DISCRIMINATIVE STIMULUS EFFECTS OF  
(±)-3,4-METHYLENEDIOXYMETHAMPHETAMINE  
(MDMA, "ECSTASY") AND (+)-LYSERGIC ACID  
DIETHYLAMIDE (LSD) IN A THREE-LEVER  
DRUG DISCRIMINATION PROCEDURE

by

Amy K. Goodwin

A Dissertation  
Submitted to the  
Faculty of The Graduate College  
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Western Michigan University  
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June 2002

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Amy K. Goodwin, Ph.D.

Western Michigan University, 2002

(±)3,4-Methylenedioxymethamphetamine (MDMA) is a common drug of abuse known as "ecstasy." Currently, MDMA is classified into the traditional drug classes as both a "stimulant" and a "hallucinogen" because it is reported to share both subjective and physiological properties of both classes. MDMA is thought to produce its psychoactive effects by acting as both a serotonin and a dopamine agonist. However, the relative importance of the serotonin and dopamine neurotransmitter systems in mediating the stimulus properties of MDMA remains unclear.

The drug discrimination assay is used to classify drugs as "similar" or "dissimilar," as well as to examine underlying neurochemical changes associated with the stimulus properties of psychoactive compounds. Two-lever drug discriminations comparing the stimulus properties of MDMA to other psychostimulants and hallucinogens have produced conflicting reports. However, Goodwin and Baker (2000) established that rats could be successfully trained to discriminate d-amphetamine, a dopamine agonist, and MDMA from saline in a three-lever drug discrimination procedure. The present study sought to train 12 rats to discriminate (+)-lysergic acid diethylamide (LSD), a serotonin agonist, and MDMA from saline in a similar three-lever procedure.

All subjects acquired the discrimination, though it appears the stimulus effects of LSD and MDMA are difficult to distinguish. This is evidenced by the difficulties establishing and demonstrating maintenance of the discrimination in the beginning stages of the study. Overall, subjects required an average of 153 training sessions in order to demonstrate adequate stimulus control.

d-Amphetamine produced only partial substitution for MDMA while the serotonin releaser, fenfluramine, did completely substitute for MDMA. Low doses of both d-amphetamine and fenfluramine given in combination substituted for MDMA at only one of the combinations. Moreover, the serotonin antagonist MDL-100907 only partially blocked the MDMA cue while the dopamine antagonist haloperidol did not produce any decrease in MDMA responding. Conversely, MDL-100907 did completely block the LSD cue.

Taken together, these results support the notion that the stimulus effects of MDMA are clearly different from those of other psychostimulants and hallucinogens, and should therefore be classified into a distinct drug class. Indeed, Nichols (1986) has proposed that MDMA and similar amphetamine analogs belong in a separate drug class, for which he has coined the term "entactogens." It also is evident that whether animals are trained to discriminate MDMA from d-amphetamine or from LSD, serotonin release is a salient feature of MDMA discrimination.

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Amy K. Goodwin

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## CHAPTER I

### INTRODUCTION

(±)-3,4-methylenedioxymethamphetamine (MDMA/Ecstasy)

#### The History of MDMA

(±)-3,4-methylenedioxymethamphetamine (MDMA) is often described as a “designer drug”, referring to compounds created by untrained chemists to be structurally similar to illegal psychoactive substances. These drugs were popular in the late 1970’s and early 1980’s because they often produced similar effects to illegal substances but were not illegal themselves. Hence, they were “designed” after existing illegal compounds to produce similar effects. In 1986 the Controlled Substances Analog Act (CSAA) was created to make this practice illegal (Grilly, 1998). However, though sometimes mistakenly reported otherwise, MDMA was not “designed” after its parent compound, d-amphetamine. Although it is structurally similar to d-amphetamine, MDMA was not initially created by lay chemists for recreational use. It is also often erroneously reported that MDMA was manufactured as a potential appetite suppressant for German soldiers during World War II (Holland, 2001; Pentney, 2001). In fact, a chemically related compound, methylenedioxyamphetamine (MDA), was synthesized by Smith Kline French in 1958 and tested as an appetite suppressant (Holland, 2001; Stafford, 1992). As a

recreational drug, MDA became popular in the mid-1960s, before MDMA was ever used recreationally (Beck & Rosenbaum, 1994; Stafford, 1992).

MDMA was originally produced by Merck, a German pharmaceutical company, in the early 1900's when their scientists were attempting to develop a vasoconstrictive drug (i.e., hydrastinin)(Holland, 2001). MDMA was one compound produced in the synthesis of hydrastinin. Indeed, when Merck filed for a patent for hydrastinine in 1912, MDMA was listed as one of the intermediate chemicals (Beck, 1997; Holland, 2001; Pentney, 2001). Thus, MDMA was included when Merck was granted the patent in 1914.

In 1953 the United States Army funded classified research, declassified in 1969, investigating the toxicity of psychoactive compounds (e.g., mescaline, MDA, MDMA) and animals (e.g., rats, mice, dogs, monkeys) (Hardman, Haavik, & Seevers, 1973; Holland, 2001; Pentney, 2001; Stafford, 1992). The research was conducted at the University of Michigan as part of a chemical warfare research project by the United States Army. As a result of these studies, MDMA was described as less toxic than MDA and no neurotoxicity was reported (Hardman et al., 1973; Pentney, 2001).

It is reported that Dr. Sasha Shulgin obtained a sample of MDMA in the early 1970's and introduced it to colleagues who were therapists, as a potential therapeutic tool (Holland, 2001). As a result, in 1976 a fair number of therapists began prescribing MDMA as an adjunct to psychotherapy (Greer & Tolbert, 1986; McDowell & Kleber, 1994), reportedly to enhance communication and "self-

examination”. One psychotherapist, Les Zeff, Ph.D., is reported to have administered MDMA during hundreds of treatment sessions without publishing any accounts (Pentney, 2001). It is during this time that MDMA was referred to as “Adam”. This in combination with the decision to not publish any reports of the use of MDMA during sessions was an attempt by Zeff to avoid alerting the media and the Drug Enforcement Agency (DEA) to the clinical use of MDMA (Pentney, 2001). It wasn’t until 1978 that the first report of the usefulness of MDMA in humans was published. Shulgin and Nichols (1978) reported that the subjective effects of MDMA included altered states of consciousness with emotional components such as empathy, acceptance, and insight. Following this report, MDMA started to gain popularity as an adjunct to psychotherapy, and as a recreational drug.

Recreational use of MDMA was on the rise in the early 1980’s and had gained the common name of “ecstasy” (Eisner, 1989). A group of chemists known as the “Boston Group” began to produce and sell MDMA and in 1981 one of these chemists branched out into Texas and began manufacturing and selling MDMA with a group of entrepreneurs (i.e., the “Texas Group”) under the name “Sassyfras” (Collin & Godfrey, 1997; Eisner, 1989; Pentney, 2001). Sassafras is an organic oil that is a precursor to the MDMA molecule (Collin & Godfrey, 1997; Eisner, 1989).

Purchases of MDMA were typically made through mail order. Indeed, this is the case historically; most substances of abuse were sold legally through mail order before

becoming illegal (Ray & Ksir, 1999). This includes amphetamine, cocaine, and even heroin.

### Legal History and Current Status of MDMA

Citing nationwide abuse and the potential health problems of MDMA, the DEA began the process of classifying MDMA as a Schedule I drug under the Controlled Substances Act of 1970 in July of 1984 (Eisner, 1989; Ray & Ksir, 1999). As part of this process, the DEA granted an appeal from a group of psychotherapists, psychiatrists, and researchers who had requested hearings on the subject of therapeutic use of MDMA. Hearings were held in Los Angeles, Kansas City, and Washington, D.C. In the meantime, the DEA invoked the 1984 Comprehensive Crime and Control Act, which allows emergency scheduling of compounds during the hearing process if the risk to the public from the substance is perceived to be high (Beck & Rosenbaum, 1994; Holland, 2001; McDowell & Kleber, 1994). In May of 1986, Francis Young, the judge overseeing the hearings, recommended to the DEA that MDMA be listed as a Schedule III drug in order to allow research and clinical applications to continue (Beck & Rosenbaum, 1994; Holland, 2001). The DEA however, ignored this recommendation and MDMA became a Schedule I compound. Following several appeals and temporary un-scheduling of MDMA, it was classified permanently as a Schedule I compound in March of 1988 and remains there today.



Once made illegal, the production and consumption of MDMA did not stop and continues to be manufactured and used recreationally. In the late 1980's the phrase "club drugs" became popular as a way to describe psychoactive substances teenagers and young adults were administering at all night dance parties, known as "rave" parties. These dance parties, which typically draw five to thirty thousand attendees, center around loud music, flashing lights, and dancing for eight to twelve hours at a time (Reynolds, 1998). Originally, rave dance parties become popular in the United Kingdom in the late 1980's but have since spread throughout the world (Collin & Godfrey, 1997; McDowell & Kleber, 1994). Several compounds have been included in a group of drugs known as "club drugs" because they are commonplace at the raves. These substances include MDMA (ecstasy), MDA (Eve), psilocybin (mushrooms), ketamine (special K), and gamma-hydroxybutyrate (GHB). Even drugs such as lysergic acid diethylamide (LSD) and alcohol have been described as "club drugs" because they are often used at these all-night parties. Although there was some general public concern as the phenomenon of all night drug festivities became more popular, it was more or less viewed as an isolated issue that only concerned those participating in the "rave" culture. Unfortunately, this was not to remain the case.

Throughout the 1990's the incidence of MDMA use has consistently increased throughout the world. It is no longer confined to the "club" environment but has become popular on university campuses and continues to gain popularity, particularly

with adolescents and young adults. For example, the National Institute on Drug Abuse (NIDA) funds an annual study conducted at the University of Michigan entitled the Monitoring the Future Study, which reports that 5.5% of 12th graders in 1999 and 8% of 12th graders in 2000 reported using MDMA within the prior year. The 2000 National Household Survey on Drug Abuse reports that 6.5 million Americans have reported trying MDMA; and the Community Epidemiology Work Group reports that in 1990 there were a mere 8 mentions of MDMA in emergency room visits but in 1999 there were 796 mentions of MDMA in emergency room visits (*NIDA Infifax*, 2002). Moreover, NIDA reported that the percentage of college students who reported having used MDMA in the past year almost increased three-fold from 1991 (0.9 percent) to 1997 (2.4 percent).

### Physiological Effects of MDMA

The cardiovascular effects reported by users and measured in the laboratory in humans typically involve increases in heart rate and blood pressure (de la Torre, Farre, & Ortuno, 2000; Mas, Farre, & de la Torre, 1999; Vollenweider, Gamma, Liechti, & Huber, 1998). There have been some isolated cases of significant hypertension reported in healthy volunteers. For example, Mat el al. (1999) administered two doses of MDMA (75 mg and 125 mg) to eight volunteers and reported that four of them were hypertensive after both doses. However, it does not appear that the scientific literature regarding human subjects as a whole supports the

notion of a linear relationship between the dose of MDMA and changes in blood pressure and heart rate (Baggot & Jerome, 2001).

Animal studies have produced conflicting results regarding cardiovascular changes. Gorder, Watkinson, O'Callaghan, & Miller (1991) reported that a dose of 20 mg/kg of MDMA in rats significantly elevated heart rate and that the increase was still evident six hours after administration. Indeed, the treatment proved to be lethal in three of the five subjects. O'Cain, Hletko, Ogden, & Varner (2000) reported that a dose of 3.0 mg/kg of MDMA significantly *decreased* the heart rate of rats. A dose of 1.0 mg/kg also decreased heart rate, though not significantly, while doses of 0.01 mg/kg and 0.1 mg/kg resulted in non-significant *increases* in heart rate. Fitzgerald & Reid (1994) reported that MDMA (1 and 10  $\mu$ m) significantly increased the heart rate of perfused, isolated, rat heart tissue *in vitro*.

Also observed in humans are thermoregulatory impairments, typically involving increases in body temperature (Liechti & Vollenweider, 2000a; Mas et al., 1999; Parrott, 2001). The environment in which the MDMA is taken can exacerbate this effect, as “ravers” typically spend long periods of time dancing within confined and crowded spaces and may consume other psychoactive substances. Indeed, a number of deaths among MDMA users participating in “raves” have been reported and attributed to hyperthermia (Cohen, 1998; Green, Cross, & Goodwin, 1995; Parrott, 2001). However, these deaths were also associated with other medical

problems, such as renal failure, cardiac arrest, liver failure, and cerebral hemorrhage (Parrott, 2001).

Thermoregulatory impairment is also reported in non-humans. Mechan, O'Shea, Elliot, Colado, & Green (2002) reported that a neurotoxic doses of MDMA (12.5 mg/kg) resulted in hyperthermia as soon as 30 minutes after administration and persisted for up to 3.5 hours in Sprague-Dawley rats. Six weeks later there was no difference in resting body temperature between MDMA and saline injected subjects. However, when subjects were exposed to a "thermoregulatory challenge" (i.e., placing the subjects in a room with a high ambient temperature,  $30^{\circ} \pm 0.5^{\circ} \text{C}$ ) the body temperature in the rats exposed to MDMA were observed to increase to higher levels than the saline control rats. Moreover, the heightened body temperature persisted much longer in the MDMA pretreated rats than in the saline pretreated rats. Other investigators (Malpass, White, Irvine, Somogyi, & Bochner, 1999) have reported that administration of non-lethal doses of MDMA (2, 5, 10 mg/kg) did not result in a significant difference in body temperature between MDMA and saline treated subjects in the Sprague-Dawley strain. However, when a Dark Agouti strain of rats was used as subjects, there was a dose-dependent increase in body temperature, and the 10 mg/kg dose was lethal in the first two subjects exposed to this dose, precluding additional testing at this dose with this strain. The Dark Agouti strain of rats are deficient in an enzyme, cytochrome P-450 2D1 (CYP2D1), which is a proposed contributor to MDMA toxicity (Malpass et al., 1999)

Other physiological changes that are reported in humans are indicative of sympathetic nervous system activation and include an increase in pupillary diameter and ocular muscle tension (Cami, Farre, & Mas, 2000; Downing, 1986), lack of appetite, jaw clenching, dry mouth, restlessness, perspiration, nausea, insomnia, tremor, fainting, blurred vision, and headache (Cohen, 1995; Curran & Travill, 1997; Davison & Parrott, 1997; Peroutka, Newman, & Harris, 1988; Schifano, 2000; Siegal, 1986; Solowij, Hall, & Nicole, 1992).

#### Neurochemical Changes Associated with MDMA Administration

There appear to be two major neurotransmitter systems involved in mediating the pharmacological effects of MDMA: serotonin and dopamine. The effects of MDMA on serotonin are typically described as both facilitating the presynaptic release (Johnson, Hoffman, & Nichols, 1986; McKenna, Guan, & Shulgin, 1991; Nichols, Lloyd, Hoffman, Nichols, & Yim, 1982; Schmidt, Levin, & Lovenberg, 1987) and blocking the reuptake (Gold & Koob, 1989). The serotonin transporter is reported to be of primary importance in MDMA's neurochemical effects (Malberg & Bonson, 2001). By occupying these transporters, MDMA prevents endogenous serotonin from binding and this results in MDMA being deposited into the presynaptic cell. Once MDMA is in the presynaptic cell it facilitates the release of serotonin. Evidence of the importance of the serotonin transporter in mediating the effects has been illustrated by Bengal, Murphy, Andrews, Wichems, Feltner, Heils,

Mossner, Westphal, & Lesch (1998). They used a transgenic serotonin transporter knockout mouse to demonstrate that MDMA-induced locomotion does not occur in the absence of serotonin transporters.

MDMA also facilitates the presynaptic release of dopamine (Johnson et al., 1986; Yamamoto & Spanos, 1988) and blocks the reuptake of dopamine (Steele, Nichols, & Yim, 1987) but to a lesser degree than that of serotonin. Additionally, the increase in dopamine release appears to be dependent upon occupation of serotonin transporters by MDMA (Nash & Brodtkin, 1991). Gudelsky & Nash (1996) reported that administration of a compound that increases serotonin synthesis before the administration of MDMA will result in an increase in dopamine release when compared to administration of MDMA alone. Moreover, administration of a serotonin-2 antagonist prior to MDMA administration blocks the increase in dopamine release (Schmidt, Taylor, Abbate, & Nieduzak, 1991). If dopamine is involved in neurotoxic effects of MDMA, 5-HT<sub>2</sub> antagonists may prove useful in preventing these effects from occurring.

### Neurotoxic Effects of MDMA

The neurotoxic effects that have been consistently reported in humans and non-humans include long-term decreases in serotonin, its metabolite 5-HIAA, serotonin transporter density, and tryptophan hydroxylase, a rate-limiting enzyme in 5-HT synthesis. A variety of methods, including neuroanatomical, neurochemical,

and functional measures have been used to examine the neurotoxic effects of MDMA on serotonin neurons in a variety of species (Ricaurte, Yuan, & McCann, 2000). Some of these techniques include immunocytochemical methods (Molliver, Berger, Mamounas, Molliver, O'Hearn, & Wilson, 1990; Ricaurte et al., 2000), silver degeneration studies (Commins, Vosmer, Virus, Woolverton, Schuster, & Seiden, 1987), imaging techniques (Holland, 1999; McCann, Szabo, Scheffel, Mathews, Dannals, Ravert, Musachio, Mertl, & Ricaurte, 1998; Scheffel, Szabo, Mathews, Finley, Dannals, Ravert, Szabo, Yuan, & Ricaurte, 1998), and anterograde transport analysis (Ricaurte et al., 2000).

Mayerhofer, Kovar, & Schmidt (2000) used High Performance Liquid Chromatography (HPLC) to examine brain tissue of rats exposed to 20 mg/kg of MDMA for ten consecutive days. They found reduced levels of serotonin in the forebrain, at both two and four weeks post-MDMA administration.

Croft, Klugman, Baldeweg, & Gruzelier (2001) employed electrophysiology to examine decreases in serotonin levels in humans. Using EEGs and EOGs, they measured subjects auditory intensity dependence function, an electrophysiological index of serotonin function (Juckel, Molnar, Hegerl, Csepe, & Karmos, 1997). Croft et. al (2001) reported that long-term MDMA users demonstrated serotonin dysfunction when compared to cannabis users and drug-naïve subjects.

Indirect evidence that serotonin transporters are involved in the neurotoxic effects of MDMA is illustrated by evidence that selective serotonin reuptake

inhibitors (SSRIs) prevent MDMA-induced serotonin depletion in rats (Schmidt, 1987; Virden & Baker, 1999). Additionally, at least three studies in humans have reported that taking an SSRI prior to common doses of MDMA reduces the positive and negative effects reported by users (Liechti & Vollenweider, 2000a;2000b; Stein & Rink, 1999). However, McCann & Ricaurte (1993) reported that four subjects who ingested the SSRI fluoxetine prior to taking MDMA stated that the subjective effects of MDMA were still experienced. Additionally, McCann, Eligulashvili, & Ricaurte (2000) reported that positron emission tomography (PET) studies document MDMA-induced neurotoxicity in nonhuman primates, as well as humans with a history of MDMA use through measurement of [ $^3$ H]McN-56 52-labeled serotonin transporter sites. McCann et. al (2000) also reported that the degree of decrease in serotonin transporter sites in humans can be correlated with the amount of MDMA exposure.

Indeed, there is little doubt that MDMA is neurotoxic and produces deficits that can be replicated across laboratories and species. What remains in question is whether these deficits correlate with functional or behavioral disruptions. Reports regarding human behavioral and cognitive correlates of MDMA neurotoxicity are conflicting. In a review of cognitive and behavioral indices of MDMA neurotoxicity in humans, Pattott (2000) reported that there are three common areas where MDMA users display deficits: impaired working memory, impaired higher order processing, and increased impulsivity. However, Parrott also reported that there were several cognitive measures where MDMA neurotoxicity did not impair performance,



including reaction time, vigilance, and verbal fluency. Indeed, Parrott reported that in some instances MDMA users had greater verbal abilities and spatial recall (Turner, Godolphin, & Parrott, 1999) when compared to non-users. McCann, Mertl, Eligulashvili, & Ricaurte, (1999) correlated significant decreases in 5-HIAA in cerebrospinal fluid (CSF) of MDMA users with cognitive performance in the same subjects. They found that MDMA users had significant deficits in measures of attention involving math tasks, complex attention and incidental learning, short-term memory and verbal reasoning. However, the MDMA users were also reported to perform the same as non-users on measures on several other cognitive tasks, including time estimation, matching to sample, and delayed recall. McCann et al., (1999) state that the differences between the groups are small, though they maintain they are significant.

Investigations of neurotoxicity and cognitive and behavioral deficits are equally conflicting in the literature regarding non-human animals. Moreover, at least one study reported that MDMA *enhanced* associative and non-associative learning in rabbits (Romano & Harvey, 1994). Regardless, both long- and short-term neurotoxicity have been documented in both humans and non-humans and should, therefore, be considered when designing and executing MDMA studies.

### Subjective Effects of MDMA

MDMA is a phenylthylamine, a structural analog of d-amphetamine and reportedly possesses both hallucinogenic and stimulant properties (Callahan & Appel, 1988; Evans & Johanson, 1986; Schechter, 1986). It is difficult to obtain reliable and valid information regarding the subjective effects of any psychoactive compound because of the inherent procedural issues required to gather data. These methods typically include self-report and anecdotal reports. Although there are inherent problems with these methods, there does seem to be a profile of the subjective effects that is common to MDMA, and distinguishable from other psychoactive compounds (Bravo, 2001).

The first published report regarding the subjective effects of MDMA came from Shulgin and Nichols (1978). Following this report, others also published accounts of the effects of MDMA in patients during therapy sessions. Among these reports, patients described elevated mood, feelings of closeness and intimacy, increased empathy, self-examination and insight, suppressed appetite, and jaw clenching (Greer & Tolbert, 1986; Shulgin & Nichols, 1978). Following the DEA's placement of MDMA as a Schedule I drug in 1985 and prior to the FDA's approval of research with MDMA in 1992, only surveys could be conducted to investigate the effects of MDMA (Bravo, 2001). These reports (Grinspoon & Bakalar, 1986; Peroutka et al., 1988; Siegal, 1986; Solowij et al., 1992) continued to include similar descriptions of the subjective effects of MDMA, but also included recreational users.

Additional aspects of the subjective effects of MDMA were added by reports of recreational users, such as alterations in color perception (Cami et al., 2000; Vollenweider et al., 1998), though hallucinations were generally only reported at very high doses (Siegal, 1986; van de Wijngaart, Braam, de Bruin, Fris, Maalste, & Verbraeck, 1999). These reports indicated that the subjective effects of MDMA shared both stimulant-like and hallucinogen-like properties. Indeed, MDMA is currently classified into the traditional drug classes as both a stimulant and a hallucinogen.

One method in which to investigate the subjective effects of psychoactive compounds under controlled conditions is the drug discrimination assay. Drug discrimination procedures are designed to systematically investigate aspects of the subjective effects of psychoactive compounds, using humans and non-humans, by providing data regarding the stimulus properties of drugs.

### The Drug Discrimination Assay

#### An Overview

The drug discrimination procedure is a popular assay used to classify the stimulus properties of psychoactive drugs. In this procedure, psychoactive drugs serve as discriminative stimuli. In the presence of the drug stimulus a specific behavior is reinforced, while in the absence of the drug stimulus another behavior is reinforced. Drug discriminations typically employ a two-lever procedure where

subjects receive a psychoactive drug or vehicle (e.g., saline). In order to receive a reinforcer (e.g., a food pellet in a food deprived subject), subjects are required to perform one behavior in the presence of the psychoactive drug (e.g., lever press on a particular lever) and a different behavior in its absence (e.g., lever press on a different lever). One method is to employ a resetting fixed-ratio schedule of reinforcement whereby a set number of *consecutive* responses (e.g., 10 lever presses) are required to obtain reinforcement. That is, a subject is required to respond on the condition-appropriate lever 10 consecutive times without responding on any other lever in order to obtain a reinforcer, if the subject responds on a different lever prior to completing the 10 consecutive responses, the ratio is reset. A subject may be said to have learned the discrimination task when condition-appropriate responding prior to the presentation of the first reinforcer is 80% or better for a predetermined number of consecutive sessions (e.g., 8 out of 10 consecutive sessions). Drug discrimination methods may also employ other schedules or reinforcement, such as fixed- or variable-interval schedules. In a review of drug discrimination methodology, Stoleran (1993) reported that not only do most studies use a fixed-ratio schedule of reinforcement but that these schedules tend to support stronger stimulus control than do other schedules.

Once a discrimination is established, other psychoactive drugs are often administered to examine whether novel drugs produce similar discriminative stimulus effects to the training drug. Generally, in a two-lever discrimination, a novel drug is

said to “substitute” if the resulting lever-pressing behavior following administration is 80% or greater on the drug-appropriate lever. That is, the subject has generalized the stimulus effects of the novel compound to the stimulus effects of the training drug. Additionally, an antagonist may be administered prior to the training drug. An antagonist is said to “block” the stimulus effects of the training drug if the resulting lever-pressing is 80% or greater on the vehicle-appropriate lever (Appel, Baker, Barrett, Broadbent, Michael, Riddle, & Van Groll, 1991).

It is sometimes the case that compounds produce asymmetrical generalization. This results when “drug A” will substitute for “drug B” but “drug B” will not substitute for “drug A”. This may result because “drug B” has multiple pharmacological actions that include those of “drug A”. Conversely, “drug A” has a relatively specific pharmacological effect that is only one aspect of the stimulus produced by “drug B”. Compounds with multiple pharmacological actions (i.e., complex stimuli) have been described as being either “conditional” or “redundant” (Grant, 1999; Mackintosh, 1974). A conditional discrimination of complex stimuli is said to require all components of the cue to be present for stimulus generalization to occur, while a redundant discrimination results in stimulus generalization when any component of the complex stimulus is presented (Grant, 1999; Jarbe, Hiltunen, & Swedberg, 1989). It has been illustrated that when drugs from different pharmacological classes are combined together for use as a discriminative stimulus, a redundant discrimination is formed (Grant, 1999; Mariathasan, Garcha, & Stoleran,

1991; Stoleran, Rauch, & Norris, 1987; Stoleran & White, 1996). That is, each compound administered individually will substitute for the training stimulus produced by the mixture of both compounds. This is important to consider when using complex discriminative stimuli and when describing asymmetrical generalizations between compounds.

In order to ensure the subjects are continuing to reliably discriminate the training conditions between testing sessions, a measure of terminal accuracy is usually employed. Terminal accuracy is generally measured by the percent of condition-appropriate responses prior to delivery of the first reinforcer during training sessions. For example, in a resetting FR 10 schedule, any number of responses may be made prior to 10 consecutive responses on the condition-appropriate lever. It is the percentage of the *total* number of responses on the condition-appropriate lever, prior to the presentation of reinforcement, which defines terminal accuracy. This measurement is generally referred to as “percent first FR responding”. Requiring subjects to maintain a percent condition-appropriate first FR above 80%-90% for the condition-appropriate lever between testing sessions is the general standard for adequate stimulus control.

### Historical Beginnings

The early beginnings of the drug discrimination procedure can be traced back to the 1830's when descriptions of state-dependent learning (SDL) were being

reported by clinicians. These clinical cases generally were descriptions of patients who were unable to recall events that occurred while they were intoxicated but once intoxicated again, where able to recall the events; or patients who were unable to recall events that occurred during some sort of paroxysmal event (Overton, Rosecrans, & Barry, 1990). In 1892, in a book entitled "*Diseases of Memory*", Ribot described the relationship between the physiological state of the body and memory recall as being mediated by the "organic sensations" occurring at the time of memory formation. These "organic senses" later became known as "interoceptive stimuli". Ribot also considered a non-drug state as equally important in the process of memory retrieval, asserting that just as memories formed during drug states are difficult to recall during non-drug states, memories formed during non-drug states are difficult to recall during drug states (Overton et al., 1990).

From 1892 up until the early 1900's, the concept of SDL appeared in writings of memory theorists and researchers, including Combe, Semon, Coriat and Prince (Overton et al., 1990). However, SDL virtually disappeared in descriptions of memory processes around 1925. This is the time when psychoanalysis emerged as a popular school of thought (Gray, 1994).

Within psychoanalysis theory, Sigmund Freud described three processes as being responsible for all aspects of behavior, including learning. The id, ego, and superego are the terms used to represent these processes. Simply put, the id was thought to be responsible for our desire for gratification, the ego for repression of

memories as a “defense mechanism” used by our unconscious in an attempt to avoid potential unpleasant emotional states, and the superego represented society’s morals. Within this framework it was thought that memory formation and learning was a result of an interaction between these three internal processes in the “unconsciousness”. The idea that memory retrieval is mediated by a portion of our unconscious “mind” left little room for SDL as a determinant of memory retrieval. Psychoanalysis became a popular school of thought and unfortunately, SDL effectively disappeared from experimental psychology and did not show up again until the late 1930’s.

In 1937, Girden and Culler reported a curare and no-drug induced dissociation in dogs. Essentially, by illustrating this curare induced dissociation Girden and Culler exemplified what is now known as drug-induced state dependent learning. It is this area of research that would become known as drug discrimination and lead the way for experimental work (Overton et al., 1990).

The first actual report of a *drug discrimination* study was by Conger (1951). He was attempting to study how alcohol affects approach and avoidance behavior in rats. He reported having trained the avoidance response under no-alcohol conditions and found a difference in later performance between no-alcohol and alcohol groups. He then noted that while there is a difference between the groups, it is possible that “the avoidance response might be due solely to a change in the animal’s condition (regardless of the direction of change) rather than to any specific effect of alcohol



because it seems likely that a change from sobriety to inebriation (or vice versa) produces a change in the animal's stimulus situation" (Conger, 1951, p.15). He then reported having successfully trained his subjects to approach under an alcohol condition and avoid under a no-alcohol condition, and vice versa. Interestingly, Conger made no statement as to how this SDL took place; he simply reported it had occurred.

In 1966, Overton reported an important development; he demonstrated that two drugs (atropine and pentobarbital) produced distinctly different stimulus effects. This led to additional studies in 1971 where Overton reported several types of drug that were discriminated from each other, and from a no-drug state. This demonstration led to what are now called "generalization gradients". That is, drugs that are part of a specific class (e.g., stimulants) will generally produce similar stimulus effects and drugs that are defined as part of different classes (e.g. a stimulant and a hallucinogen) will produce distinctly different stimulus effects (Barry, 1974). This is demonstrated by the general finding that the administration of drugs from the same class will result in drug-appropriate responding and the administration of drugs from different pharmacological classes will produce responding on the lever correlated with the absence of drug.

During the 1960's and 1970's many researchers began using variations in the approach/avoidance procedure to study the stimulus effects of drugs. The T-maze was one such procedure. In this assay a subject is reinforced for entering one side

following drug administration and the opposite side in the absence of the drug. In this way, the rate suppressing effects that may influence the interpretation of single response go-no go procedures (i.e., approach/avoidance) were minimized. Another major change was the use of operant lever-pressing behavior, reported to be sensitive to even lower doses of drugs than the T-maze (Kubena & Barry, 1969). Then in 1975 the fixed ratio (FR) schedule of reinforcement was introduced into drug discrimination research by Colpaert and his colleagues. The advantage of the FR schedule is a higher level of accuracy (Overton et al., 1990). Most recently, researchers have begun using drug versus drug discriminations, and three-lever procedures where subjects are trained to discriminate between two drugs and a vehicle. It appears that these are more sensitive assays with which to study the stimulus effects of psychoactive drugs (Stolerman, 1993), particularly those with multiple pharmacological actions (Baker & Taylor, 1997).

The popularity and use of the drug discrimination assay has grown profoundly since its inception. Researchers have used the assay to study a vast array of centrally acting compounds. Indeed, an entire database of published drug discrimination studies (i.e., the drug discrimination bibliography) since 1970, created by I.P. Stolerman, can be accessed via the World Wide Web.

### Contributions of the Drug Discrimination Assay

The drug discrimination assay is used by researchers for a variety of purposes. These include describing the stimulus properties of an assortment of psychoactive substances, investigating underlying neurochemical processes of these substances, indexing abuse potential of drugs, investigating the time-course of drug effects, and aiding new drug development (Colpaert, 1986;1999; Holtzman, 1990).

Using the drug discrimination procedure, the stimulus properties of drugs can be described as “similar” or “dissimilar”. This is useful when classifying psychoactive substances (i.e., stimulants, hallucinogens, etc.). This sort of elementary description of drugs is a useful starting point for investigation of centrally acting compounds.

Although there are assays which are traditionally used to measure the abuse potential of drugs (e.g., self-administration, progressive ratio schedules), the drug discrimination assay may be used to examine aspects of the abuse potential of drugs (Glennon, 1991). For example, a novel compound substituting for cocaine in a drug discrimination procedure may be interpreted as a preliminary indication of abuse potential. One might then pursue the issue in assays specifically designed to measure abuse potential.

The drug discrimination assay also makes important and unique contributions to the development of new therapeutic agents (Meert & Awouters, 1990). For example, it has been reported that (5-HT<sub>2</sub>) antagonists may produce anti-anxiety and

antidepressant properties (Glennon, 1991). Potential therapeutic compounds may be identified by testing them for their antagonist properties in 5-HT<sub>2</sub> agonist discrimination designs.

The drug discrimination assay may also be used to examine the underlying neurochemical mechanisms involved in producing the stimulus properties of drugs. This is an important aspect for potential treatments of drug overdose, as well as the development of agents to treat drug addiction. A compound that is a known antagonist for a specific receptor site that blocks the stimulus effects of an abused drug may be helpful in treating drug addiction, or aid in the development of agents to treat an overdose.

#### Limitations of the Drug Discrimination Assay

A major limitation of the drug discrimination assay is the extensive amount of time required to conduct a single study. A typical two-lever procedure generally requires 20-40 training sessions while the more complex three-lever design has been reported to require between 80-100 sessions (Baker & Taylor, 1997; Goodwin & Baker, 2000; Overton, 1978). The time requirement to conduct drug discrimination research is further prolonged by the methodological consideration that once the discrimination is established, at least one training day for each stimulus condition must occur between test days. Thus, in a two-lever discrimination there are at least two training days between test sessions and this is increased to a minimum of three

training days for the three-lever design. Essentially, a typical two-lever study requires a minimum of nine to twelve months to complete while the more complex three-lever design requires between twelve and fifteen months.

A second methodological consideration in drug discrimination research is data interpretation. The nature of the drug discrimination assay assures that regardless of the stimulus properties of a particular compound, subjects will respond. As noted above, a novel drug is said to “substitute” if the resulting lever-pressing behavior following administration of a novel compound is 80% or greater on the drug-appropriate lever. Conversely, an antagonist is said to have “blocked” the stimulus effects of the training drug if the resulting lever-pressing is 80% or greater on the vehicle-appropriate lever. However, data interpretation when lever-pressing is between 20% and 80% on a single lever, what is referred to as “partial substitution” or “partial blockade”, is problematic. Some have suggested that this may be interpreted as the subject responding on a sort of continuum of drug effect where the percent responding represents a description of the level of drug effect but this has not been experimentally tested (Colpaert, 1988) and so cannot be assumed to be true.

#### Drug Discrimination and MDMA

As described earlier, the drug discrimination assay is a useful tool in drug abuse research and is used extensively with psychoactive drugs of abuse, particularly to classify drugs as similar or dissimilar. It is critical to examine MDMA in the drug

discrimination assay because of its reported complex and relatively unknown mechanisms of action. Indeed, MDMA has been extensively investigated using the traditional two-lever drug discrimination method; however, these reports have yielded conflicting results. There are relatively few studies that have examined the stimulus properties of MDMA using a three-lever procedure.

In 1987 Schechter reported that MDMA produced stimulus control in rats trained to discriminate MDMA from saline using a FR 10 schedule of food reinforcement. He also reported that MDMA substituted for itself in a dose-dependent manner. Prior to this published account, MDMA had only been used to test for substitution in studies examining the stimulus control of other drugs. For example, Schechter (1986) reported that MDMA substituted for the serotonin agonist fenfluramine, the indirect dopamine agonist *l*-cathinone, and the serotonin agonist tetrahydro- $\beta$ -carboline (THBC). Schechter concluded that the discriminative stimulus effects of MDMA were probably mediated via indirect-acting dopaminergic agonist properties, and also by acting upon a subtype of serotonin receptor, probably the 5-HT<sub>2</sub> receptor subtype. This conclusion was supported by other studies as well (Broadbent, Appel, Michael, & Ricker, 1992).

However, there are conflicting reports regarding the substitution of MDMA for the known dopamine agonist, d-amphetamine. With d-amphetamine (2.0 mg/kg) as the training drug, Evans and Johanson (1986) reported that MDMA (3.0 mg/kg) substituted for d-amphetamine in pigeons responding under a FR 30 schedule of

reinforcement. However, this study used a total of only three subjects. Glennon and Young (1984) also reported that MDMA (2.25 mg/kg) would also generalize to d-amphetamine (1.0 mg/kg) in rats trained to discriminate d-amphetamine from saline. Subjects were trained to respond under a VI 15s schedule of reinforcement. Glennon et. al used four subjects, but only three completed the test session at the dose which produced complete substitution (2.25 mg/kg). Conversely, Oberlander and Nichols (1988) reported that MDMA (2.63 mg/kg) did *not* substitute for d-amphetamine (1.0 mg/kg) in rats (n=14) trained to discriminate d-amphetamine from saline under a FR 50 schedule of reinforcement. In addition, Oberlander et. al also reported that d-amphetamine (1.2 mg/kg) *did* substitute for MDMA (1.75 mg/kg) when MDMA was the training stimulus, but was disruptive in seven of the thirteen rats tested. Schechter (1989) trained eight rats to discriminate MDMA (1.5 mg/kg) from saline under a FR 10 schedule of reinforcement. He reported that d-amphetamine (0.8 mg/kg) only partially substituted for MDMA. Glennon and Misenheimer (1989) also reported that d-amphetamine (1.0 mg/kg) only partially substituted for MDMA (1.5 mg/kg) in rats (n=4) trained to discriminate MDMA from d-amphetamine under a VI 15s schedule of reinforcement.

There are also conflicting reports of the substitution of MDMA to serotonin agonists, such as LSD and fenfluramine. Schechter (1986) reported that MDMA (2.0 mg/kg) substituted for the training drug fenfluramine (2.0 mg/kg) in rats (n=10) trained to discriminate fenfluramine from vehicle under a FR 10 schedule of

reinforcement. Schechter (1998) also reported that LSD (0.12 mg/kg) substituted for MDMA (1.5 mg/kg) in a similar procedure. There is also at least one study that reported nearly complete (78%) substitution of LSD (0.16 mg/kg) for MDMA (1.75 mg/kg) (Oberlander & Nichols, 1988) in a two-lever procedure. Norfenfluramine (1.4 mg/kg), another 5-HT agonist, has also been reported to substitute for MDMA (1.5 mg/kg) in rats (Schechter, 1989). However, Callahan and Appel (1988) reported that MDMA did not substitute for LSD in rats.

Although there are relatively few studies which have examined the stimulus properties of MDMA in more complex discriminations (i.e., three-lever or drug vs. drug), Evans, Zacny, and Johanson (1990) trained five pigeons to discriminate various doses of d-amphetamine, fenfluramine, and saline using a three-lever procedure and a FR 30 schedule of reinforcement. In the three subjects tested, they reported that MDMA substituted for d-amphetamine in two of the subjects and for fenfluramine in the third subject. Baker and Taylor (1997) also examined the stimulus properties of MDMA in two separate, three-lever drug discrimination experiments. In the first, rats were trained to discriminate d-amphetamine (1.0 mg/kg), mescaline (12.5 mg/kg), and saline under a FR 20 schedule of reinforcement. Stimulus generalization tests with (+)-MDMA did not result in complete substitution for d-amphetamine, but resulted in mostly saline-appropriate responding, with some responding on the mescaline-appropriate lever. Administration of (-)-MDMA produced 78% mescaline-appropriate responding. In the second experiment, Baker



et. al trained rats to discriminate d-amphetamine (1.0 mg/kg), LSD (0.08 mg/kg), and saline. They reported that neither isomer of MDMA substituted for d-amphetamine, and actually produced some responding on the LSD-appropriate lever, though not enough to represent full substitution.

In an attempt to further investigate the stimulus properties of MDMA, Goodwin and Baker (2000) trained rats to discriminate between d-amphetamine (1.0 mg/kg), MDMA (1.5 mg/kg), and saline under a FR 10 schedule of reinforcement. We reported that all the subjects were able to learn the discrimination and that d-amphetamine produced dose-dependent increases in d-amphetamine-appropriate responding and MDMA produced dose-dependent increases in MDMA-appropriate responding. We also demonstrated that the administration of LSD resulted in dose-dependent increases in MDMA-appropriate responding with almost complete substitution for MDMA (i.e., 78% MDMA-appropriate responding) at the two highest doses tested (0.08 and 0.16 mg/kg). The administration of cocaine resulted in dose-dependent increases in d-amphetamine-appropriate responding, with complete substitution at the highest dose tested (10 mg/kg). Additionally, fenfluramine substituted for MDMA, as did both isomers of MDA, while the 5-HT<sub>2</sub> antagonist pirenperone only partially blocked the stimulus effects of MDMA. These results are particularly significant because they are definitive in that subjects were able to learn to discriminate between MDMA and d-amphetamine, leaving little doubt that

although MDMA is structurally related to d-amphetamine, the discriminative stimulus effects of these two compounds are clearly different.

It also appears that in that procedure, MDMA served as a redundant discriminative stimulus rather than a conditional stimulus. That is, the compound stimulus effects produced by MDMA acting as both a serotonin and dopamine agonist were differentiated from the relatively specific stimulus effects d-amphetamine produced by its actions as a dopamine agonist. This is further supported by the fact that serotonin agonists (e.g., LSD, fenfluramine, and MDA) produced dose-dependent increases in MDMA-appropriate responding. If MDMA had served as a conditional stimulus it would follow that all components of the MDMA stimulus would have to be present for the stimulus effects of a drug to substitute for the stimulus effects of MDMA in this procedure and drugs acting relatively specifically as serotonin agonists would not substitute for MDMA in this procedure. It also follows then, that rats trained to discriminate MDMA from a serotonin agonist (e.g., LSD) in a similar procedure would generalize the stimulus effects of dopamine agonists (e.g., d-amphetamine) to MDMA.

It has also been suggested that subjects rely on the most salient part of compounds with complex stimulus properties in order to discriminate them (Wood, Lal, Yaden, & Emmett-Oglesby, 1985). It appears that the serotonergic effects of MDMA become more salient in maintaining stimulus control when animals are trained to discriminate both d-amphetamine and MDMA. Thus, it is also possible that

the dopaminergic effects of MDMA could become more salient if rats were trained to discriminate between MDMA and a serotonergic compound, such as LSD. If this is the case, then one would expect d-amphetamine to substitute for MDMA in these animals.

### Rationale for the Present Study

The present study was designed to further investigate the discriminative stimulus effects of MDMA. Specifically, since MDMA is classified as both a stimulant and a hallucinogen, and it has been established that rats can discriminate between d-amphetamine and MDMA in a three-choice procedure, the present study sought to determine if rats could be trained to discriminate between a hallucinogen (i.e., LSD) and MDMA in a similar three-lever procedure. Additionally, if such a discrimination could be established in rats, this study sought to determine what pharmacological actions of MDMA were most salient in maintaining stimulus control in these animals.

## CHAPTER II

### METHODS

#### Subjects

Twelve experimentally naïve Sprague-Dawley rats (Harlan Breeding Laboratories, Indianapolis, IN) approximately 60 days old at the beginning of the study, and weighing between 250 and 300 g were used as subjects. Subjects were individually housed in plastic shoebox cages in a colony maintained on a 12-h light (0700 to 1900)/12-h dark cycle, at relatively constant temperature (20-22 ° C) and humidity levels (50-60%). In the home cages, subjects were allowed free access to water while food intake was restricted to maintain body weights between 85% and 90% of their free feeding weights for the duration of the study. The experimental protocol was reviewed by the Institutional Animal Care and Use Committee of Western Michigan University and subjects were maintained according to the general principles of animal husbandry outlined by the National Institutes on Drug Abuse.

#### Materials

All training and testing procedures were conducted in eight standard operant test chambers (MED Associates, Inc., Georgia, VT) measuring 30 x 31 x 24 cm, maintained in sound- and light-attenuating cubicles. The chambers were equipped

with three retractable levers on the front panel, a 28-V house light located on the rear panel, and a food pellet delivery mechanism located above the center lever.

The (±)-MDMA, (+)-LSD, d-amphetamine sulfate, and fenfluramine hydrochloride were obtained from the National Institute on Drug Abuse (Rockville, MD). The MDL-100,907 was generously donated by Dr. Meltzer (Vanderbilt University) and the haloperidol was obtained from Sigma (St. Louis, MS). The (±)-MDMA, (+)-LSD, d-amphetamine, and fenfluramine were dissolved in 0.9% bacteriostatic sodium chloride and administered intraperitoneally 15 min prior to training and testing sessions. The MDL-100,907 was dissolved in sterile water and administered 30 min prior to testing. The haloperidol was dissolved in sterile water with drops of lactic acid added until the drug went into solution, and administered 45 minutes prior to testing. All drugs were administered in an injection volume of 1 ml/kg.

### Training Procedures

An autoshaping procedure was used for the first week of the experiment. Subjects received between 5 and 6 one hour sessions where no substances were administered, no levers were present in the chamber, and food pellets were delivered on a fixed-time 60 sec (FT 60") schedule of food delivery. Subsequently, errorless discrimination training was employed where only the condition-appropriate lever was present for alternate 20 min training sessions of saline and each drug condition. This

was continued until each subject was exposed to at least four errorless training sessions for each of the three conditions. During the errorless training sessions a fixed-ratio one (FR 1) schedule of reinforcement was used.

Following the errorless training procedures, all three levers were presented and discrimination training began with a FR 1 schedule of reinforcement during daily 20 min training sessions. The ratio was gradually increased to 10 as responding became stable. The terminal schedule of reinforcement was a resetting FR 10. That is, reinforcement was contingent on 10 consecutive responses on the condition-appropriate lever, responses on any other lever reset the response counter and reinforcement was not delivered until 10 consecutive responses were made on the condition-appropriate lever. Subjects were able to obtain an unlimited number of reinforcers during the 20 min training sessions. With the administration of MDMA, half of the subjects were reinforced for responses on the left lever and half were reinforced for responses on the right lever. The conditions were reversed for the administration of LSD. Under saline conditions, all subjects were reinforced for responses on the center lever. In order to reduce the effects of olfactory cues between animals in the operant chambers, all levers were wiped with isopropyl alcohol between training sessions (Extance and Goudie, 1981). Additionally, the order in which subjects were run during the daily sessions was altered randomly. Training sessions were conducted 6 days a week at approximately the same time each day.

### Testing Procedures

Once subjects met a pre-determined criterion for discrimination (80% of responses on the condition-appropriate lever prior to the delivery of the first reinforcer for at least 8 out of 10 consecutive training sessions), testing procedure were implemented. Test sessions were similar to training sessions except that no reinforcers were delivered and the animals were removed from the chambers immediately upon completion of 10 consecutive responses on any lever. Test sessions were conducted once or twice per week in place of training sessions, provided that during training sessions the animals maintained 80% or better condition-appropriate responding prior to the delivery of any reinforcers under each stimulus condition.

Stimulus generalization tests were conducted with three doses of each training drug (MDMA 0.375-1.5 mg/kg; LSD 0.02-0.08 mg/kg), d-amphetamine (0.50-2.0 mg/kg), fenfluramine (0.50-2.0 mg/kg), and the combination of fenfluramine (0.25-0.50 mg/kg) and d-amphetamine (0.25-1.0 mg/kg). Antagonist tests were conducted with the 5-HT<sub>2</sub> antagonist MDL-100,907 (0.0325-0.50 mg/kg) in combination with the training dose of MDMA (1.5 mg/kg) and in combination with the training dose of LSD (0.08 mg/kg). Additionally, haloperidol (0.1-0.4 mg/kg) was administered in combination with the training dose of MDMA.

## Data Analysis

Initially, all subjects received the same stimulus for training sessions and the criterion for acquisition of the discrimination was 80%-100% stimulus appropriate responding prior to the delivery of the first reinforcer for eight out of ten consecutive training sessions. However, following the initiation of dose-response curves for the training drugs (i.e., MDMA and LSD) it appeared that stimulus control was inadequate. Despite methods used to reduce olfactory cues, it is possible that some subjects were using residual olfactory cues, or some other cue, during training sessions. Therefore, rather than continuing to administer the same training stimulus to all subjects, the three stimulus conditions were varied across subjects starting at training sessions 137. In this way, any residual olfactory cues were not reliable prompts for identification of the lever correlated with the presentation of reinforcement during any given training session. Following this change, all subjects were required to again meet the criterion for discrimination. The number of sessions to criterion, both before and after this procedural change, is presented for visual analysis.

A dose-response curve was generated for each compound tested in order to depict the percent of total responses on each lever for each dose tested, as well as the overall response rate at each dose. A group mean was calculated for each measure at each dose. Only the data from subjects who emitted at least ten responses during



testing sessions were included to calculate the percentage of responses on each lever data. The data from all subjects were used to calculate response rates. A one-way analysis of variance (ANOVA) was used to analyze stimulus generalization data and response rate for each compound tested. Complete stimulus generalization was defined as at least 80% responding on either the ( $\pm$ )-MDMA or (+)-LSD appropriate lever. Complete stimulus blockade was defined as at least 80% responding on the saline-appropriate lever. For compounds that produced stimulus generalization or stimulus blockade, nonlinear regression analyses were calculated to determine ED<sub>50</sub>s.

## CHAPTER III

### RESULTS

#### Sessions to Criterion

All 12 subjects acquired the discrimination of LSD and MDMA. Following the discovery that stimulus control was not reliably maintained when the training doses were assessed during initial stimulus generalization tests, the stimulus conditions during the daily training sessions were varied for individual subjects. For example, subjects 1 through 6 may have received MDMA as the training stimulus for the same training session that subject 7 through 12 were administered LSD. Figure 1 illustrates the number of sessions to meet the preset criterion for discrimination for each individual subject, both before and after this procedural change. The mean number of sessions for the subjects to initially meet the discrimination was 49 (SEM = 3.18, range = 35 to 68). Upon resuming training with differing training stimuli among subjects, the mean of the total number of sessions to criterion (i.e., including the original number to meet the preset criterion) was 153 (SEM = 2.86, range = 145 to 174).

### Stimulus Generalization

Figure 2 represents the results of stimulus generalization tests with MDMA (0.375-1.5 mg/kg). There were dose-dependent increases in MDMA-appropriate responding with virtually no LSD-appropriate responding across doses. The  $ED_{50}$  for MDMA was 0.97 mg/kg (95% confidence intervals: 0.2488-1.694). There were significant dose-dependent increases in the percentage of MDMA-appropriate responding across doses [ $F_{(3,43)}=21.18$ ,  $p<.0001$ ] but no differences in response rates across doses [ $F_{(3,43)} = 1.09$ ,  $p>.05$ ].

The dose-response data for LSD (0.02-0.08 mg/kg) are presented in Figure 3. The  $ED_{50}$  for LSD was 0.038 mg/kg (95% confidence intervals: 0.006-0.223). There were significant dose-dependent increases in LSD-appropriate responding [ $F_{(3,43)}=23.77$ ,  $p<.0001$ ] with no MDMA-appropriate responding at 0.04 mg/kg nor the training dose, 0.08 mg/kg. However, there was a small amount (13%) of MDMA-appropriate responding at 0.02 mg/kg of LSD. Response rates did not differ across doses [ $F_{(3,43)} = 0.19$ ,  $p>.05$ ].

Figure 4 represents generalization tests with d-amphetamine (0.25-2.0 mg/kg). There were significant dose-dependent increases in MDMA-appropriate responding [ $F_{(4,22)}=3.1$ ,  $p<.05$ ] and dose-dependent decreases in saline-appropriate responding. However, d-amphetamine did not completely substitute for MDMA at any of the doses tested. There was MDMA-appropriate responding at both the 1.0 mg/kg and

2.0 mg/kg doses with the greatest percentage of MDMA-appropriate responding at 2.0 mg/kg, (60% MDMA-appropriate responding), the highest dose tested. There were significant dose-dependent decreases in the rate of responding, with severe rate suppression at the highest dose tested [ $F_{(4, 29)} = 3.028$ ,  $p < .05$ ]. Six animals were tested at the 2.0 mg/kg dose, the highest dose administered, and only three completed the test. Due to the severe suppression of response rate, higher doses were not tested. Two subjects were administered the 0.25 mg/kg dose for later comparison to the combinations of d-amphetamine and fenfluramine.

The results of substitution tests with fenfluramine (0.25-2.0 mg/kg) are presented in Figure 5. As illustrated, there are significant dose-dependent increases in MDMA-appropriate responding [ $F_{(5,33)} = 19.41$ ,  $p < .001$ ], with complete substitution at the highest dose tested, 2.0 mg/kg. The  $ED_{50}$  for fenfluramine was 1.42 mg/kg (95% confidence intervals: 0.927-1.91). There was not a significant amount of LSD-appropriate responding at any of the doses tested, though at the 1.0 mg/kg dose there was 20% LSD-appropriate responding. There were no significant differences across doses with respect to response rate [ $F_{(5,33)} = 1.01$ ,  $p > .05$ ].

Figure 6 illustrates the percentage of MDMA-appropriate responding with doses of d-amphetamine (0.25, 0.50, 1.0 mg/kg) and fenfluramine (0.25, 0.50 mg/kg) administered in combination. This was done to examine the possibility that these compounds may have synergistic effects. Included in Figure 6 is the percentage of MDMA-appropriate responding for fenfluramine and d-amphetamine administered

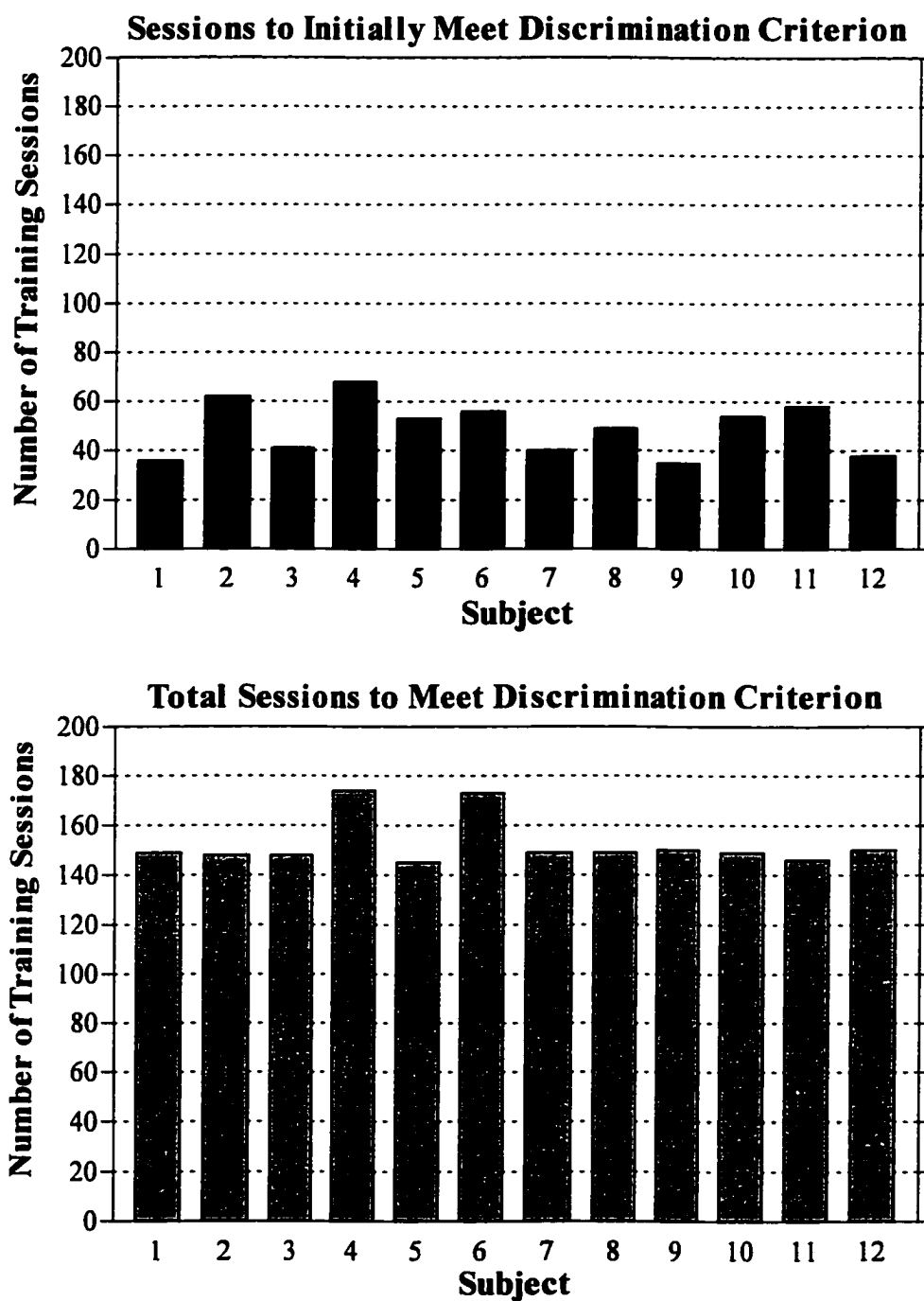
alone such that they may be compared to the administration of the combinations of both drugs. One combination (0.50 mg/kg d-amphetamine and 0.50 mg/kg fenfluramine) resulted in complete substitution for the MDMA cue. However, the differences in MDMA-appropriate responding across the doses of d-amphetamine (0.25-1.0 mg/kg) was not quite significant [ $F_{(2,17)}=3.63$ ,  $p=.051$ ] when combined with 0.25 mg/kg of fenfluramine. The combination of 1.0 mg/kg d-amphetamine and 0.25 mg/kg fenfluramine produced nearly complete substitution for MDMA (79%). The differences in MDMA-appropriate responding was not significant when 0.50 mg/kg of fenfluramine was combined with d-amphetamine (0.25-1.0 mg/kg) [ $F_{(2,17)}=1.83$ ,  $p<.05$ ]. There was virtually no LSD-responding at any of the dose combinations tested. There were dose-dependent decreases in rate of responding, with the lowest response rates occurring at the 1.0 mg/kg of d-amphetamine in combination with 0.25 and 0.50 mg/kg of fenfluramine. Although the response rate was significantly suppressed [ $F_{(5, 17)} = 3.86$ ,  $p>.05$ ], all subjects completed the test sessions at all the dose combinations tested.

The administration of MDL-100907 (0.03125-0.50 mg/kg) prior to the training dose of MDMA (1.5 mg/kg) did not produce dose-dependent decreases in MDMA-appropriate responding (Figure 7). In fact, MDL-100907 administration produced the lowest percentage of MDMA-appropriate responding (28% MDMA-appropriate responding) at 0.0625 mg/kg, but this was neither the lowest nor the highest dose of MDL-100907 tested. The percentage of MDMA-appropriate

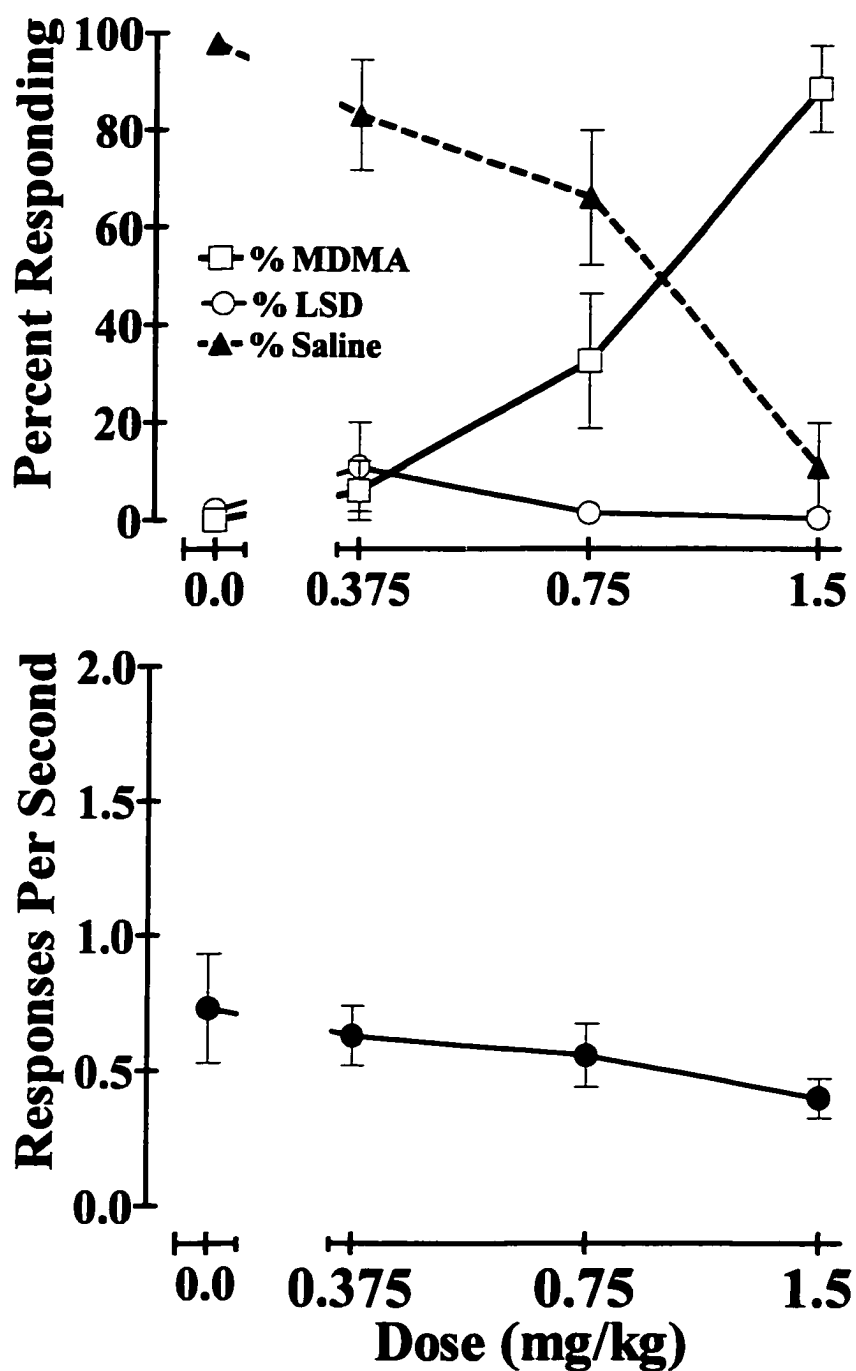
responding when .0625 mg/kg of MDL-100907 was given prior to MDMA was significantly different from the percentage observed when vehicle was given prior to MDMA [ $F_{(5,41)}=2.76$ ,  $p<.05$ ]. Although the rate of responding decreased in a dose-dependent fashion, the difference across doses was not significant [ $F_{(5,41)} = .47$ ,  $p>.05$ ].

Conversely, the administration of MDL-100907 (0.03125-0.50 mg/kg) in combination with the training dose of LSD (0.08 mg/kg) resulted in complete blockade of the LSD stimulus [ $F_{(5,30)}=26.81$ ,  $p<.0001$ ] at all of the doses of MDL-100907 tested (Figure 8). LSD-appropriate responding occurred in only one subject at one dose (0.25 mg/kg). The differences in response rate across doses was not significant [ $F_{(5,30)} = 1.08$ ,  $p>.05$ ].

The administration of haloperidol (0.1-0.4 mg/kg) in combination with the training dose of MDMA (1.5 mg/kg) did not result in any decreases in MDMA-appropriate responding (Figure 9). That is, only MDMA-appropriate responding was observed at all of the doses of haloperidol tested. Haloperidol also produced a significant decrease in response rate [ $F_{(3,11)} = 4.85$ ,  $p<.05$ ]. Due to the advanced age of the subjects, only three subjects were tested on the haloperidol and MDMA combination.

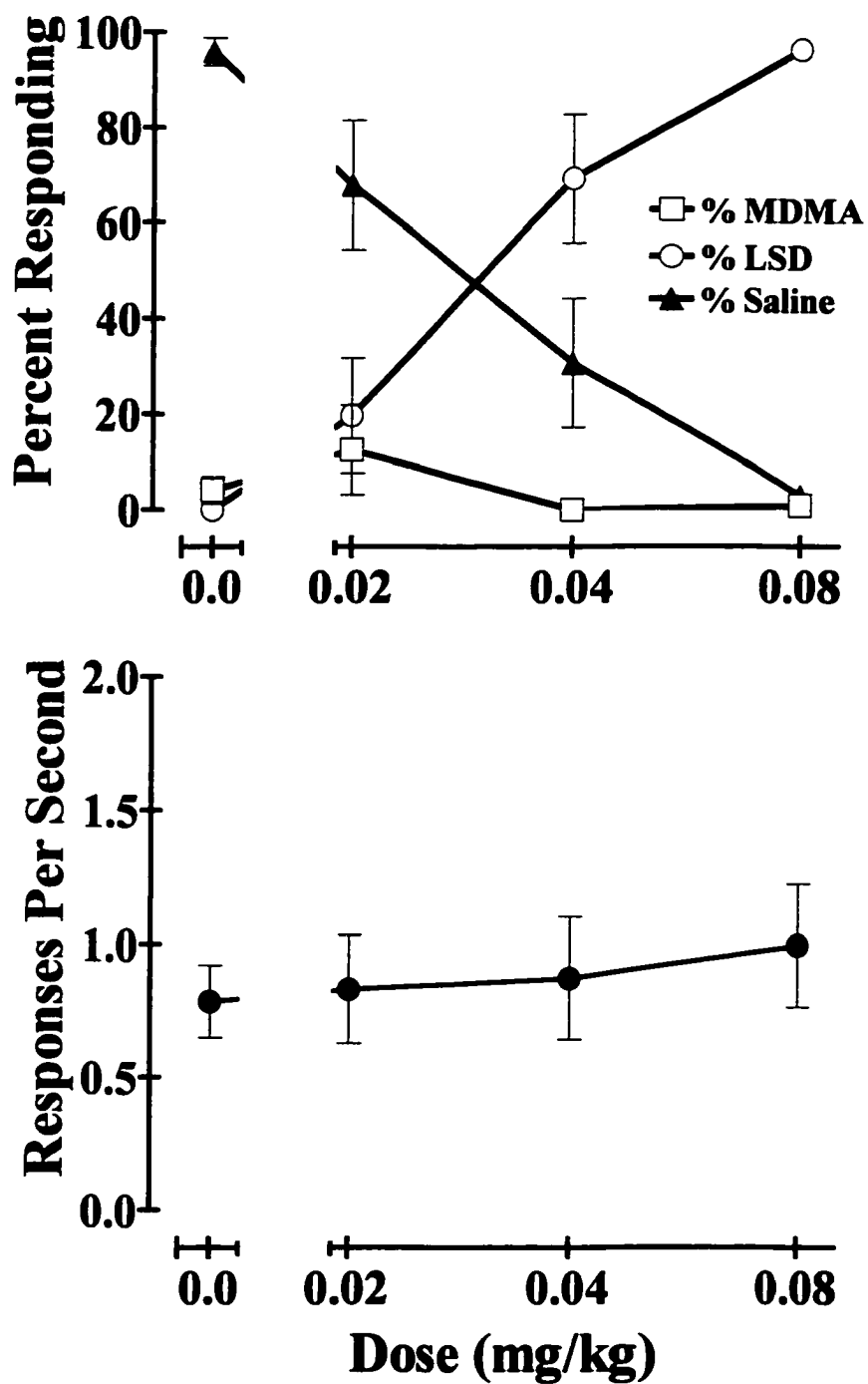


**Figure 1.** Illustration of the Number of Sessions Required to Establish the LSD/MDMA/Saline Discrimination (N=12).

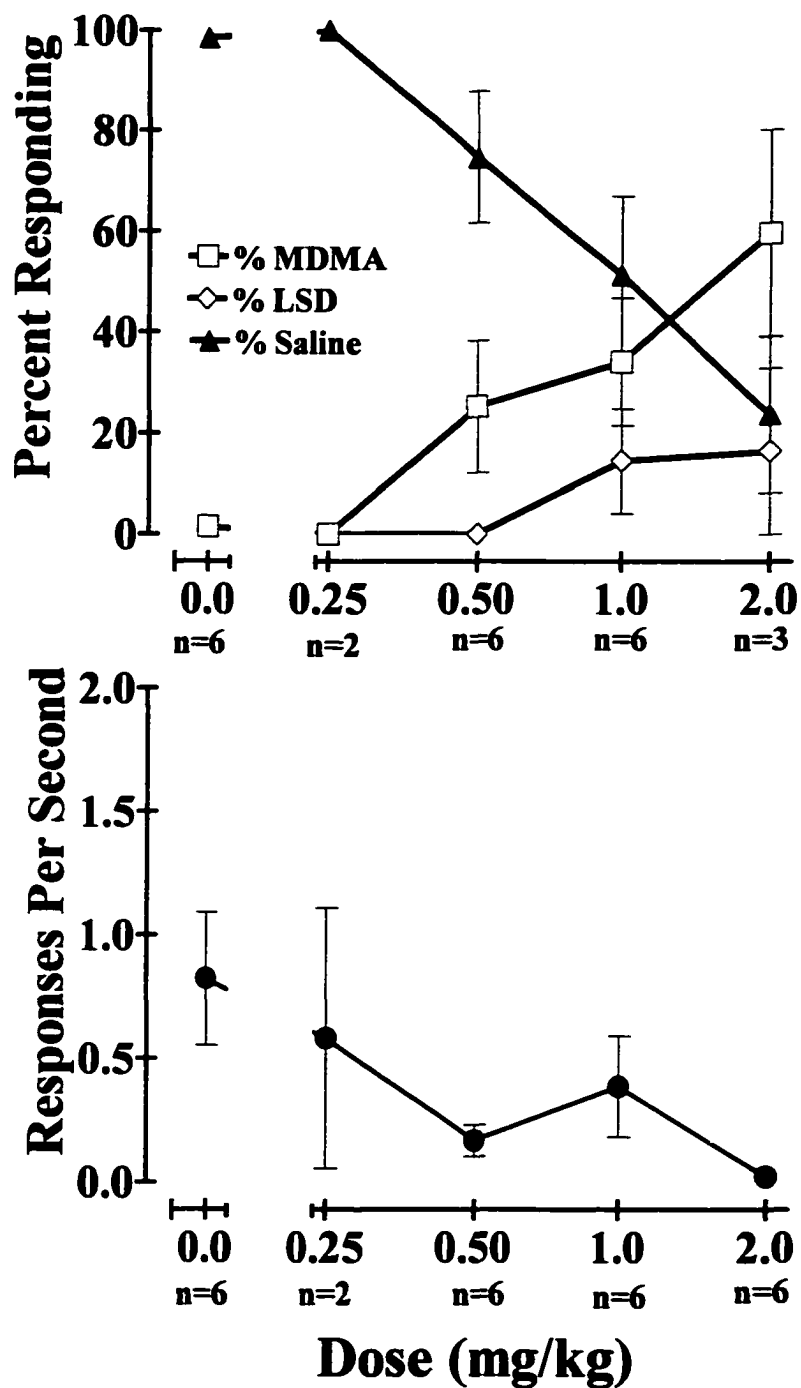


**Figure 2.** Results of Stimulus Generalization tests with MDMA (n=11).

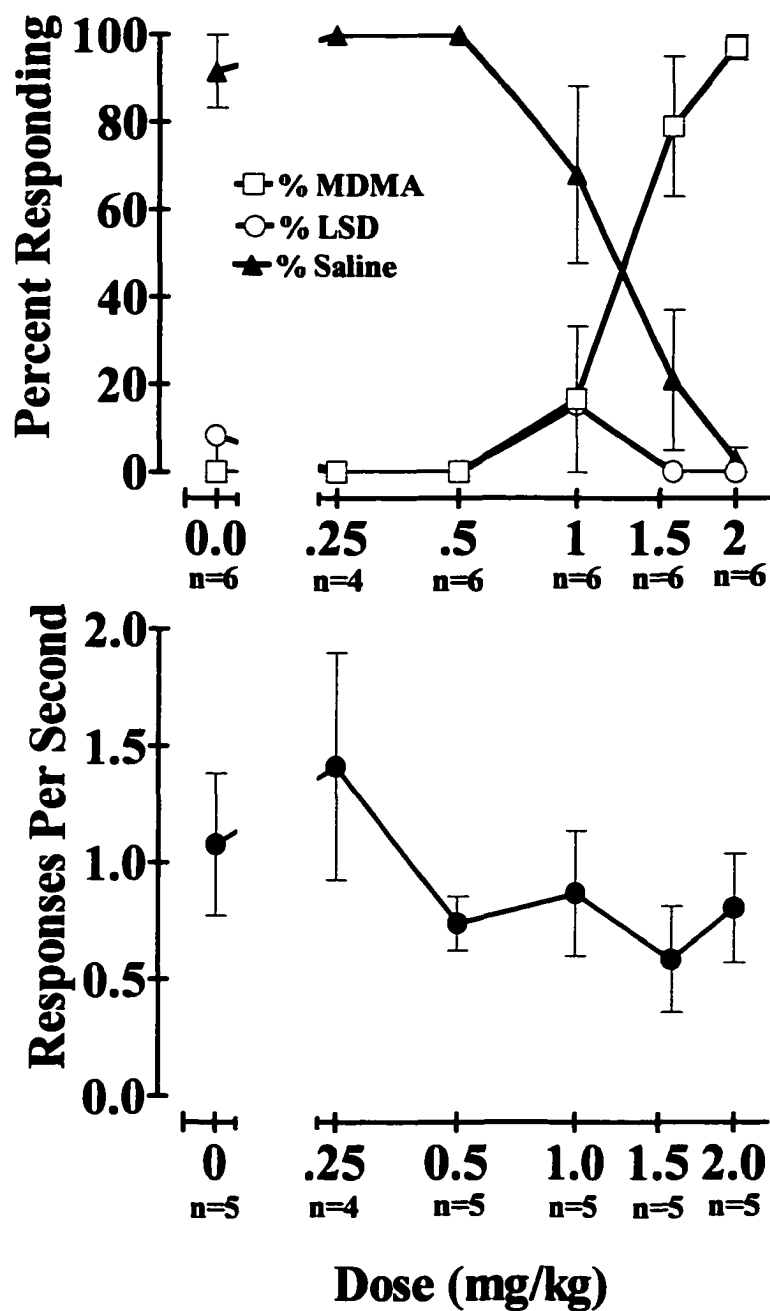




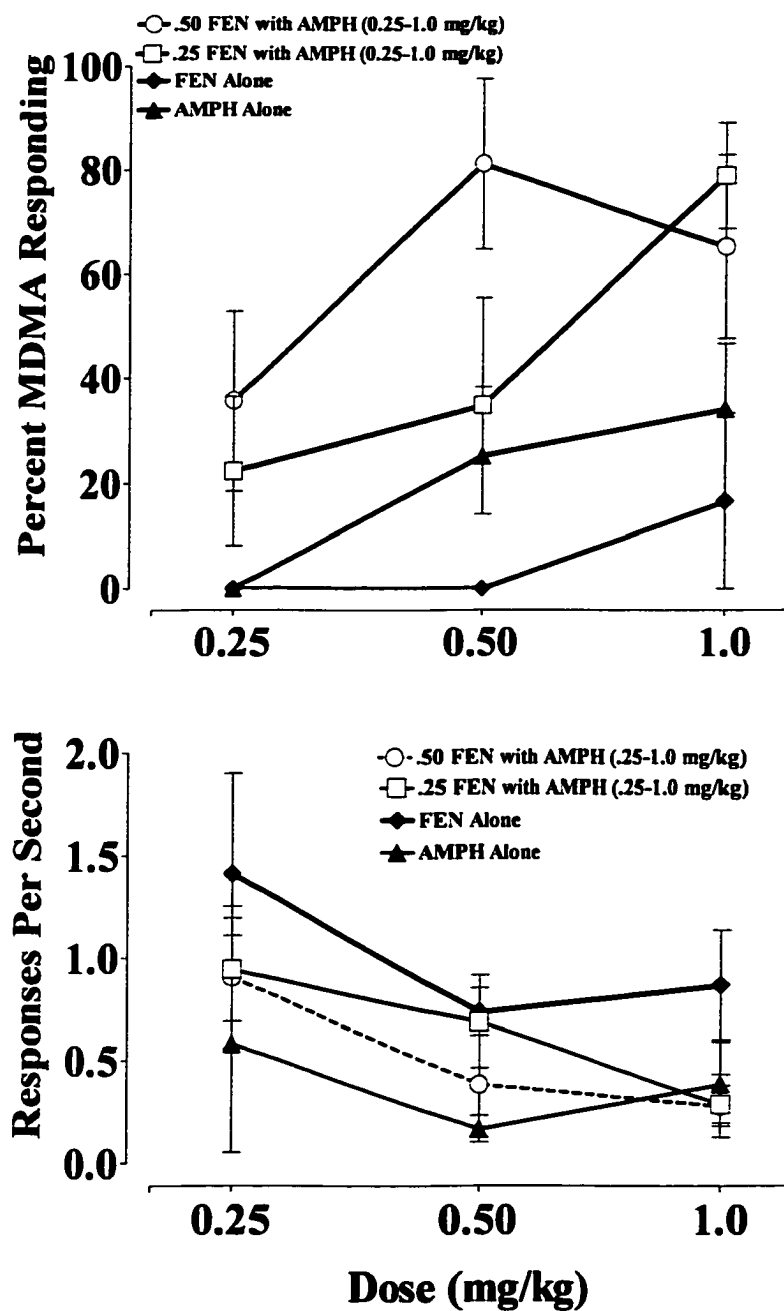
**Figure 3.** Results of Stimulus Generalization Tests with LSD (n=11).



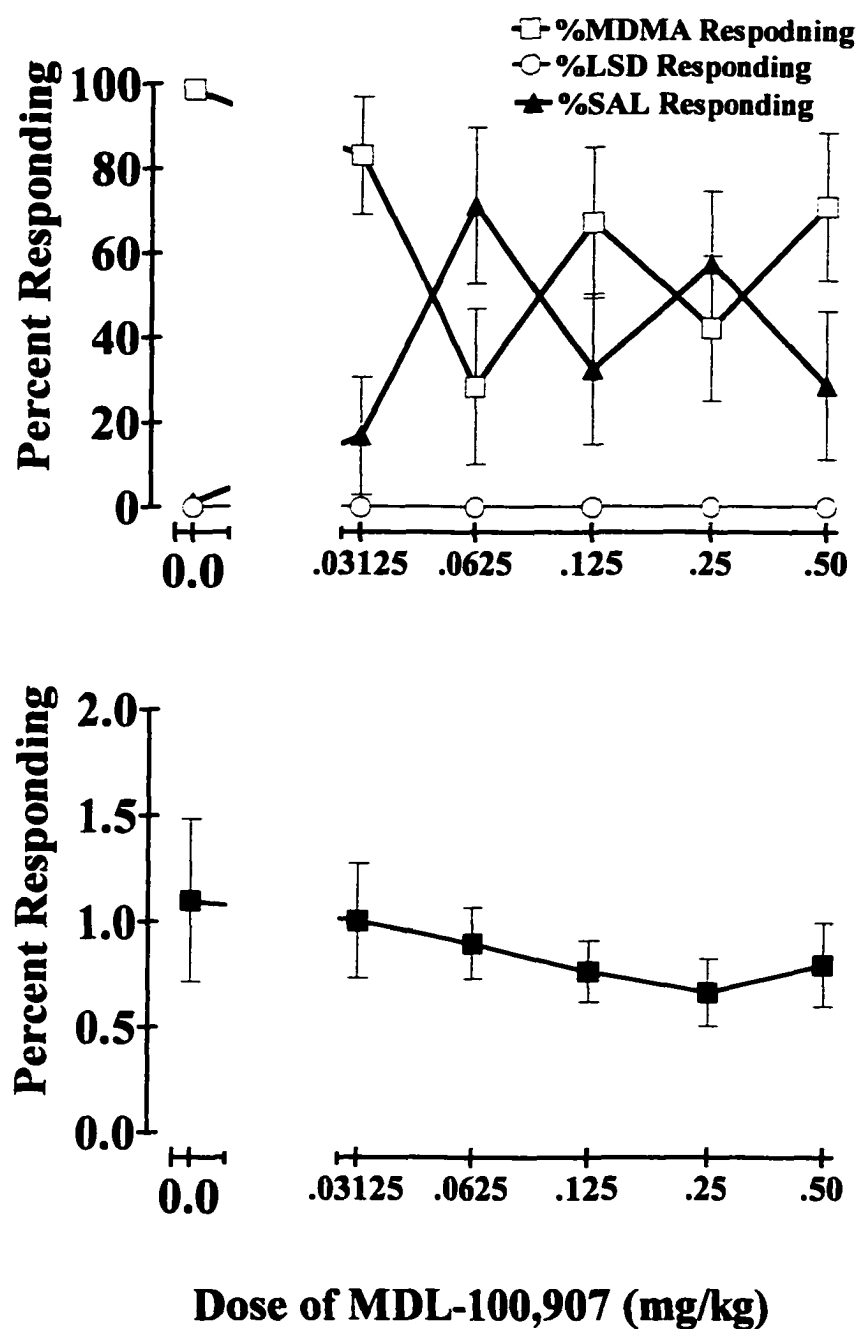
**Figure 4.** Results of Stimulus Generalization Tests with d-Amphetamine.



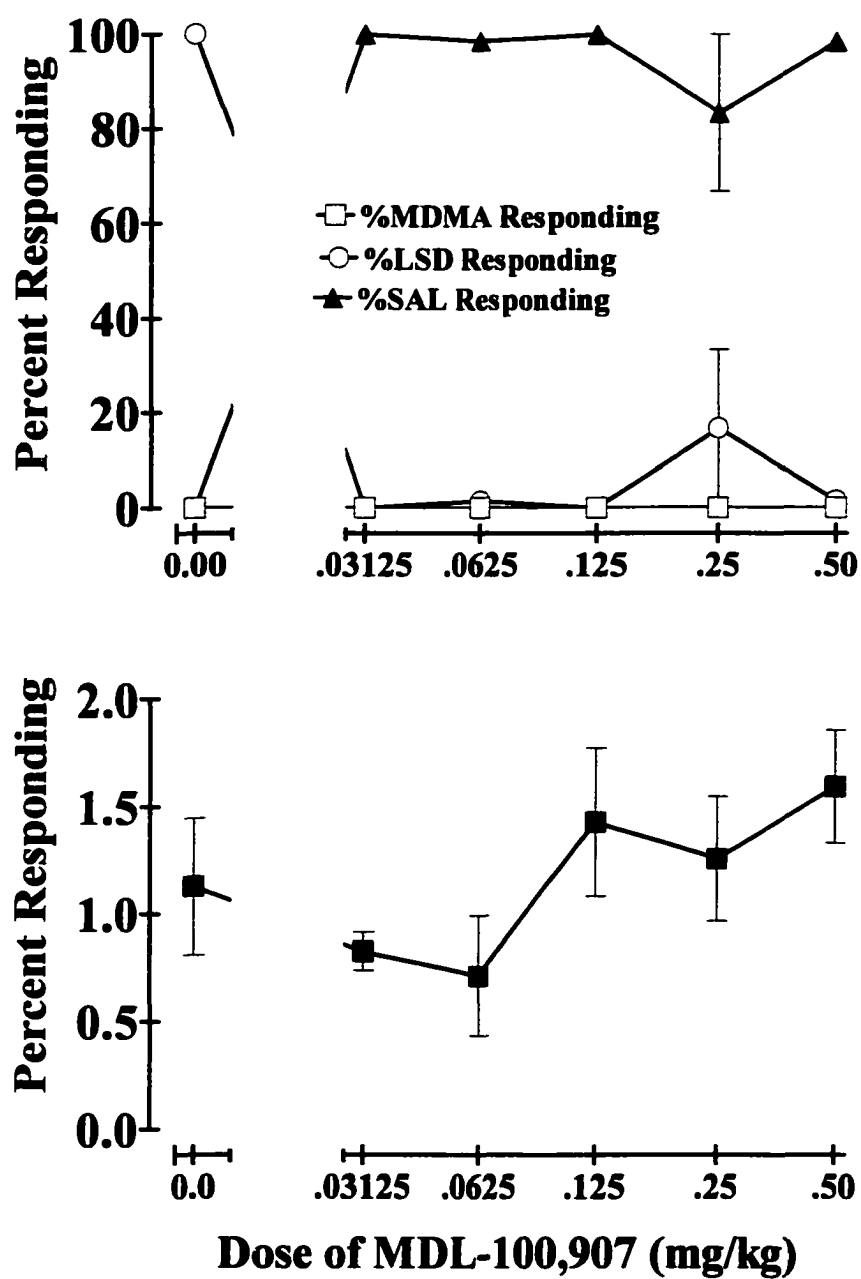
**Figure 5.** Results of Stimulus Generalization Tests with Fenfluramine.



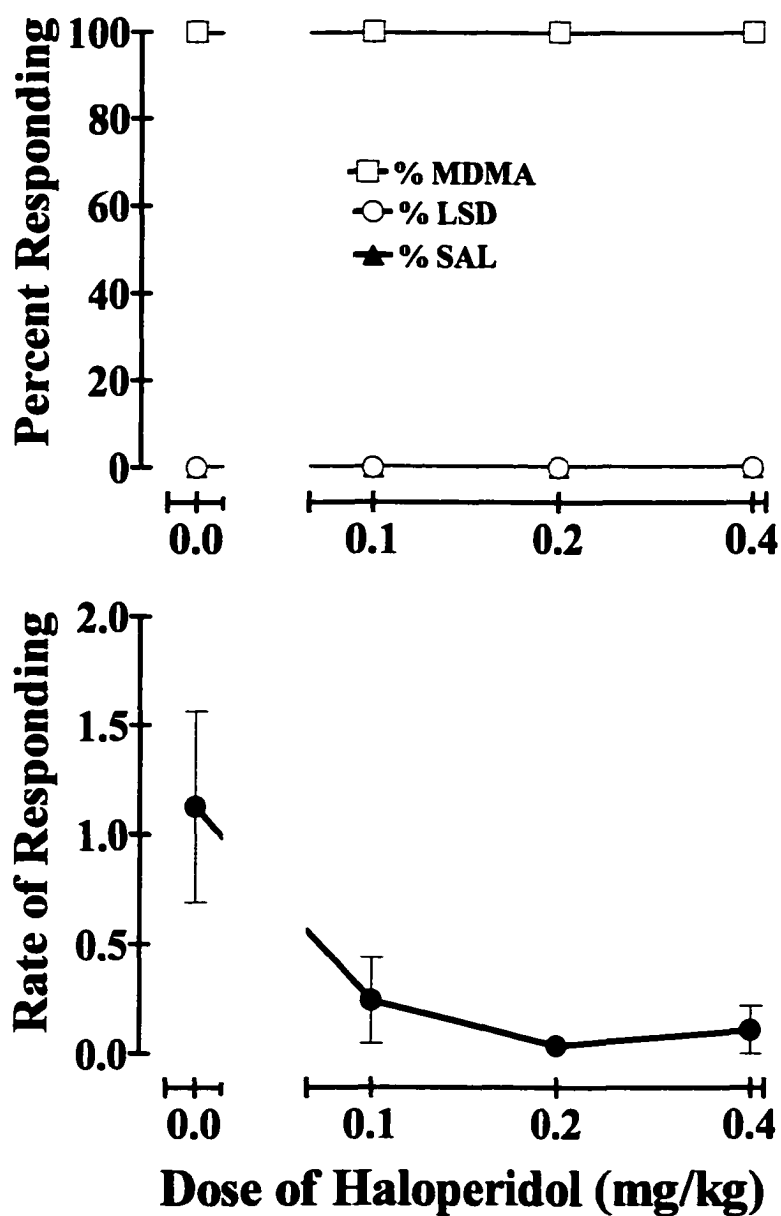
**Figure 6.** Results of Stimulus Generalization Tests with d-Amphetamine and Fenfluramine Given in Combination (n=6), d-Amphetamine Alone (n=6) and Fenfluramine Alone (n=6).



**Figure 7.** Results of Antagonism Tests with MDL-100907 Administered Prior to MDMA (n=7).



**Figure 8.** Results of Antagonism Tests with MDL-100907 Administered Prior to LSD (n=7).



**Figure 9.** Results of Antagonism Tests with Haloperidol Administered Prior to MDMA (n=3).

## CHAPTER IV

### DISCUSSION

The present results support the notion that, despite its classification as both a stimulant and a hallucinogen, MDMA produces complex stimulus effects that are distinctly different from those of other psychostimulants and hallucinogens. Indeed, it has been proposed that MDMA and similar amphetamine analogs belong to a separate drug class called “etactogens” (Nichols, 1986). Previous studies have concluded that the stimulus properties of MDMA are mediated through both serotonergic and dopaminergic actions (Glennon, Higgs, Young, & Issa, 1992; Malberg & Bonson, 2001; Schechter, 1989), though the relationship between these actions and the resulting stimulus effects is not well understood. The relative importance of dopaminergic vs. serotonergic actions in maintaining stimulus control by MDMA appears to depend on the drug discrimination methods employed. Moreover, conflicting results from previous drug discrimination studies with MDMA are likely due to methodological differences among laboratories.

It is well established that the stimulus properties of d-amphetamine are primarily mediated via changes in dopamine (Goudie, 1991; Ho & Huang, 1975; Nielsen & Jepsen, 1985; Woolverton, 1984; Yokel & Wise, 1976). It is also well-documented that the stimulus effects of LSD are primarily mediated through actions on serotonin (Bonson, Buckholtz, & Murphy, 1996; Cameron & Appel, 1973;



Glennon, Rosecrans, & Young, 1982; Sadzot, Baraban, Glennon, Lyon, Leonhardt, Jan, & Titeler, 1989; Trulson, Ross, & Jacobs, 1976). Baker and Goodwin (2000) recently demonstrated that rats could be trained to dissociate the effects of d-amphetamine from those of MDMA in a three-lever assay. One may conclude that, *in that procedure*, serotonergic actions became a more salient feature of MDMA's discriminative stimulus effects relative to its dopaminergic actions. This is further supported by the observation that the administration of other serotonin agonists (i.e., LSD and fenfluramine) resulted in dose-dependent increases in MDMA-appropriate responding, while cocaine, a dopamine agonist, produced full substitution for d-amphetamine. Therefore, it is possible that the dopaminergic effects of MDMA could become more salient if rats were trained to discriminate between MDMA and a serotonin agonist such as LSD. However, the present results do not support this hypothesis.

In the present study, rats were successfully trained to discriminate between LSD and MDMA. All subjects were required to meet the discrimination criterion twice because stimulus control was not adequately maintained following initial dose-response determinations with the training drugs. Although the experimental chambers were wiped with isopropyl alcohol between groups, it is possible that stimuli from previous subjects in the same chamber were contributing to the initial development of stimulus control. Thus, the three stimulus conditions (i.e., MDMA, LSD, and saline) were varied across subjects so that any olfactory cues present in the

experimental chambers were not reliable prompts for identifying the appropriate lever during training sessions. Following this change, subjects were again required to meet the criterion for discrimination.

It is apparent from the total number of sessions required to meet the discrimination criterion (80% of responses on the condition-appropriate lever prior to the delivery of the first reinforcer for at least 8 out of 10 consecutive training sessions) that the stimulus effects of LSD and MDMA are difficult to distinguish. Indeed, a typical two-lever discrimination can require between 30 and 60 training sessions to establish while the present study required a mean of 153 sessions to establish and reliably maintain the discrimination. Additionally, although all 12 subjects met the criterion for discrimination, one of them did not maintain the discrimination and was never tested for stimulus generalization. None-the-less, all the subjects did meet the criterion for stimulus control and both training doses produced substitution in the 11 subjects tested.

Although subjects did learn to discriminate between MDMA and LSD, d-amphetamine failed to substitute for MDMA, while fenfluramine produced full stimulus generalization to MDMA. This supports previous reports that the serotonin releaser fenfluramine (Goodwin & Baker, 2000; Schechter, 1986) and its metabolite, norfenfluramine (Schechter, 1989) substitute for MDMA, but not for LSD (Callahan & Appel, 1988). The present findings also support the notion that the stimulus effects of MDMA and d-amphetamine are clearly different (Goodwin & Baker, 2000;

Nichols, 1986; Schechter, 1997). Moreover, it appears that even in animals trained to discriminate a serotonin agonist (LSD) from MDMA, 5-HT release is a more salient feature of MDMA's discriminative stimulus effects than is dopamine release.

Because d-amphetamine produced partial substitution for MDMA, it is possible that these effects could be potentiated by lower doses of fenfluramine. Therefore, d-amphetamine and fenfluramine were tested in combination. Neither the 0.25 mg/kg nor the 0.50 mg/kg dose of fenfluramine resulted in any MDMA-appropriate responding when given alone, while the 1.0 mg/kg dose of amphetamine produced only 34.5% MDMA-appropriate responding when administered alone. It was hypothesized that combining low doses of both compounds would result in a synergistic effect and produce generalization to the MDMA stimulus. Only one combination of d-amphetamine and fenfluramine completely substituted for MDMA (0.50 mg/kg d-amphetamine and 0.50 mg/kg fenfluramine). Interestingly, when the dose of d-amphetamine was increased to 1.0 mg/kg and combined with 0.50 mg/kg of fenfluramine, the amount of MDMA-appropriate responding decreased from 80% to 65%. Additionally, 1.0 mg/kg d-amphetamine and 0.25 mg/kg fenfluramine produced nearly complete substitution (79%) for the MDMA stimulus.

MDL-100907, a 5-HT<sub>2</sub> antagonist, has been reported to block both MDMA stimulated dopamine release and long-term 5-HT deficits associated with MDMA (Schmidt, 1992). However, in the present study, MDL-100907 had differential effects when administered in combination with MDMA. No clear linear relationship

was observed between MDL 100,907 dose and MDMA-appropriate responding. Indeed, as is evident in the Figure 7, the percentage of drug lever-responses at all of the MDL-100907 doses was highly variable among subjects. At one dose of MDL-100907 (0.0625 mg/kg) there was only 27% MDMA-appropriate responding, though this was neither the highest nor the lowest dose tested. At a lower dose (0.03125 mg/kg) MDMA appropriate responding was 83%, while a dose of 0.125 mg/kg produced 67% MDMA-appropriate responding. Moreover, at the highest dose of MDL-100907 tested (0.50 mg/kg) there was 70% MDMA-appropriate responding. This supports previous reports that multiple 5-HT receptor subtypes may be involved in producing the stimulus effects of MDMA. Specifically, 5-HT<sub>1</sub> and 5-HT<sub>2</sub> antagonists (Glennon et al., 1992; Schechter, 1989) have been reported to decrease MDMA-appropriate responding and 5-HT<sub>3</sub> antagonists have been reported to completely block the MDMA cue (Glennon et al., 1992).

Conversely, MDL-100907 completely blocked the LSD cue at all of the doses tested, including the dose which resulted in the lowest percentage of MDMA-appropriate responding (0.0625 mg/kg). This supports previous reports that the stimulus properties of LSD are mediated primarily through its actions on 5-HT<sub>2</sub> receptors (Bonson et al., 1996; Cameron & Appel, 1973; Glennon et al., 1982; Sadzot et al., 1989; Trulson et al., 1976).

Haloperidol, a dopamine antagonist, failed to decrease MDMA-appropriate responding at any of the doses tested. This further supports the notion that the

stimulus effects of MDMA were not primarily mediated via its actions on dopamine receptors in this procedure. However, this is contrary to a previous report that haloperidol decreased the subjective, euphoric effects of MDMA when given prior to MDMA in fourteen healthy volunteers (Liechti & Vollenweider, 2000).

Three major conclusions have been gained from the present study. First, the stimulus properties of MDMA consist of multiple actions, and are distinctly different from the discriminative stimulus effects of both LSD and d-amphetamine. Thus, one may argue it is not appropriate for MDMA to be classified into the traditional hallucinogen and stimulant drug classes. Second, whether animals are trained to discriminate MDMA from d-amphetamine or from LSD, serotonin release is a salient feature of MDMA discrimination. However, the failure of MDL-100907 to fully block MDMA discrimination in the present study suggests that other 5-HT receptor mediated actions are also involved. A combination of a serotonin and dopamine antagonists may block the stimulus effects of MDMA in this procedure. Unfortunately, due to the advanced age of the subjects further tests could not be conducted. Thus, a third major conclusion is that although three-lever drug discrimination procedures provide a more sensitive tool with which to investigate drugs with compound stimulus properties, the time required to train such a discrimination limits the number of compounds that can be assessed for stimulus generalization and stimulus antagonism. Indeed, although the present study established that the discriminative stimulus effects of LSD and MDMA can be

differentiated, the amount of information obtained regarding pharmacological actions mediating the stimulus properties of MDMA was limited by the extensive amount of time required to establish and maintain stimulus control in this procedure.

**Appendix**  
**Institutional Animal Care and Use Committee**  
**(IACUC) Protocol Clearance**

**WESTERN MICHIGAN UNIVERSITY  
INVESTIGATOR IACUC CERTIFICATE**

Title of Project: Application of differential outcomes to a three-choice drug discrimination utilizing LSD and MDMA

The information included in this IACUC application is accurate to the best of my knowledge. All personnel listed recognize their responsibility in complying with university policies governing the care and use of animals.

I declare that all experiments involving live animals will be performed under my supervision or that of another qualified scientist. Technicians or students involved have been trained in proper procedures in animal handling, administration of anesthetics, analgesics, and euthanasia to be used in this project.

If this project is funded by an extramural source, I certify that this application accurately reflects all procedures involving laboratory animal subjects described in the proposal to the funding agency noted above.

Any proposed revisions to or variations from the animal care and use data will be promptly forwarded to the IACUC for approval.

       Disapproved             Approved        X   Approved with the provisions listed below

Provisions or Explanations:

see attached sheets

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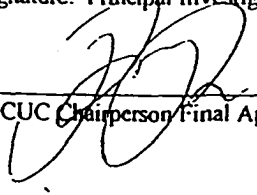
  
IACUC Chairperson

3/2/00  
Date

Acceptance of Provisions

Signature: Principal Investigator/Instructor

Date

  
IACUC Chairperson Final Approval

3/28/00  
Date



## WESTERN MICHIGAN UNIVERSITY

March 15, 2000

Bruce Bejcek, Ph.D.  
IACUC Chair  
Biological Sciences Department  
Western Michigan University


Dear Dr. Bejcek:


In regards to the IACUC committee's concerns for protocol (00-02-02) entitled "Application of differential outcomes to a three-choice drug discrimination utilizing LSD and MDMA":

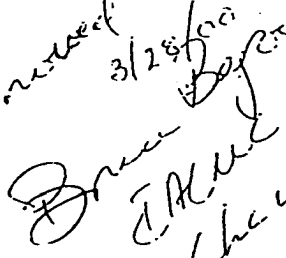
- 1) Animals will be exposed to training sessions six days per week. Drug will not be administered for more than two consecutive training days. Test sessions will occur once per week on average. That is, one training day for each condition (i.e., LSD, MDMA, and saline) will separate test days. Thus, test sessions will occur approximately once every four days. The total time duration for the study will be approximately 52 weeks.
- 2) The principal investigator will monitor animals for one hour after testing for complications. Additionally, a full time animal care staff monitors the health of all animals on a daily basis. If complications should arise where an animal is in obvious pain and/or distress, CO<sub>2</sub> will be used to euthanize the animal. Additionally, a veterinarian is available for consultations.
- 3) The database search included the years 1960 through the present.

Thank you for your time and consideration.

Sincerely,

  
Lisa Baker, Ph.D.  
Psychology Department  
Western Michigan University  
Kalamazoo, MI 49008

  
Amy Goodwin, MA  
Psychology Department  
Western Michigan University  
Kalamazoo, MI 49008

Approved 3/28/00  
  
Bruce Bejcek  
IACUC  
Chair

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