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## Assessment of the Stimulus Properties of MDA and MDMA Stereoisomers in a LSD-Saline-Amphetamine Discrimination

Michele Marie Taylor

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**ASSESSMENT OF THE STIMULUS PROPERTIES OF MDA AND MDMA  
STEREoisomers in a LSD-Saline-Amphetamine  
Discrimination**

by

**Michele Marie Taylor**

**A Thesis  
Submitted to the  
Faculty of The Graduate College  
in partial fulfillment of the  
requirements for the  
Degree of Master of Arts  
Department of Psychology**

**Western Michigan University  
Kalamazoo, Michigan  
June 1996**

# ASSESSMENT OF THE STIMULUS PROPERTIES OF MDA AND MDMA STEREOISOMERS IN A LSD-SALINE-AMPHETAMINE DISCRIMINATION

Michele Marie Taylor, M.A.

Western Michigan University, 1996

This study employed a three-choice drug discrimination procedure in order to further delineate the discriminative stimulus properties of the stereoisomers of 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA). Eight male Sprague-Dawley rats were trained to discriminate amphetamine (1.0 mg/kg) and LSD (.08 mg/kg) from saline in a three-lever, food reinforced (sweetened condensed milk) drug discrimination procedure. A fixed-ratio (FR) 20 schedule with a reset condition for incorrect responses was employed. When criteria (85% over 10 consecutive sessions) were met, (+)-MDA, (-)-MDA, (+)-MDMA (.31, .63, 1.25 mg/kg) and (-)-MDMA (.88, 1.75, 3.5 mg/kg) were tested for substitution. All of these compounds produced a greater percentage of responding on the LSD lever than on the amphetamine lever. These results suggest that the discriminative stimulus properties of both MDMA and MDA isomers resemble those of LSD more closely than those of amphetamine. It is suggested that the use of a three-lever discrimination procedure affords a greater degree of precision than the traditional two-level assay in the assessment of the complex stimulus properties of these designer drugs.

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## CHAPTER I

### INTRODUCTION

#### The History of MDMA

Ecstasy, often called 'E', 'ADAM' or 'XTC' is known chemically as 3,4-methylenedioxymethamphetamine (MDMA). MDMA, a phenylisopropylamine, is a structural analog of amphetamine. It is also chemically related to the psychedelic drug 3,4-methylenedioxymethamphetamine (MDA or the "love drug"). By adding a methyl group to MDA, the "kitchen chemists" of the late 1960s wanted to produce a new drug with the effects of its parent but of shorter duration. However, MDMA is not a new drug. It was first synthesized by Merck, a German pharmaceutical firm in Darmstadt Germany in 1912 (Beck & Rosenbaum, 1994) and patented in 1914 (McDowell & Kleber, 1994). "MDMA was not, as is sometimes believed, initially intended as an appetite suppressant, but was originally developed as a parent compound" (McDowell & Kleber, 1994, p. 127). When the anorectic effects of the drug were discovered, it was proposed as an appetite suppressant for soldiers during the First World War (Redhead, 1993). However, it never achieved clinical applicability for this indication. The United States Army experimented with the compound during the 1950s. Since the obtained information was made public in the early 1970's interest in the compound waned for many years.

"MDMA was first used recreationally by humans in the late 1960s" (McDowell

& Kleber, 1994, p. 127). It was "discovered" by New Age seekers who valued its capacity to induce feelings of well-being and connection to others. Beginning in 1976, a small number of therapists on both coasts began to utilize MDMA for similar reasons. MDMA was believed to be beneficial as a therapeutic adjunct on the predication that it facilitated communication, acceptance, and disinhibition. Shulgin and Shulgin (1991) recount the story of a psychologist they named "Adam," who is purportedly the "father" of MDMA use as an adjunct to psychotherapy. Despite their belief in MDMA's efficacy, therapists were reluctant to publish any preliminary findings, fearing that such efforts would only hasten the criminalization of the then legal drug and block further research (Eisner, 1989; Seymour, 1986). It was not until the late 1970s that the first published pharmacological investigation of MDMA in humans appeared. Shulgin and Nichols (1978) described how MDMA evoked an easily controlled altered state of consciousness, with emotional and sensual overtones. These properties made it promising to therapists and tempting, eventually, to the curious public as well (Shulgin & Nichols, 1978). The ongoing therapeutic use remained unknown to much of the public with the exception of a slowly burgeoning population of recreational users. Then, when distribution patterns changed and the media discovered the drug in the mid 1980s, the recreational market proliferated.

### The Emergence of the Contemporary Psychedelic Subculture

The recreational market for MDMA slowly expanded in the early 1980s. This was a period during which a group of chemists in the Boston area, called the "Boston Group," had commenced production in 1976 (Beck & Rosenbaum, 1994). In anticipation of

enjoying greater profits by expanding efforts to meet ever increasing demands for MDMA, the Southwest distributor for the Boston Group put together his own operation with the financial backing of some friends from Texas (Beck & Rosenbaum, 1994). As Beck and Rosenbaum (1994) described:

The "Texas Group" quickly became the largest and most intrepid MDMA distribution network in the nation. The Texas distributors used blatant promotional tactics, circulating posters announcing "Ecstasy parties" at bars and discos with MDMA billed as a "fun drug" which was "good to dance to." Interestingly, the drug was available over the counter at bars and convenience stores. Such billing appealed to young adults (p. 19).

"For this group, the drug's capacity to induce feelings of connection, as well as a psychomotor agitation that can be pleasurably relieved by dancing, make it an ideal party drug" (McDowell & Kleber, 1995, p. 128). Not surprisingly, recreational use of the drug at dance clubs exploded. Recently, MDMA's popularity has been inextricably linked with the rise of the rave phenomenon. "Raves" are all-night dance marathon parties popular in England since the 1980s, which have found their way to the United States and the rest of the world (McDowell & Kleber, 1995). The recreational use of MDMA at all-night "raves" has been implicated in a number of deaths in Britain. It has been postulated (Randall, 1992) that the reported deaths were associated with the setting at such "raves." For instance, the crowded conditions, high temperature and loud noise may have enhanced the toxicity of the drug. Further, dehydration has been implicated as a variable which may modulate the toxicity of MDMA (Green, 1995). Indeed, on a hot day in the summer of 1992 a number of deaths, as many as 15, were correlated with the use of MDMA in clubs which had turned off their water supplies in an effort to maximize profits



by selling bottled water (McDowell & Kleber, 1995). According to the British National Poisons unit, the fatalities were the result of a form of heatstroke, caused by the effects of MDMA which were intensified by dehydration and prolonged, vigorous dancing in hot, stifling settings. The English government has since mandated a continuous water supply at all clubs. Concomitantly, the reports of MDMA related deaths had stopped. These alarming reports coupled with the considerable literature regarding the long-term neurotoxic effects (Ricaurte et al., 1985; Schmidt et al., 1986) of the drug prompted the campaign to criminalize MDMA.

### The Criminalization of MDMA

The rampant use of MDMA attracted the attention of Texas Senator Lloyd Bentsen. He petitioned the Food and Drug Administration and the compound was placed in Schedule I of the Controlled Substances Act on an emergency basis on July 1, 1985 (McDowell & Kleber, 1994, p. 128). This occurred prior to the previously scheduled series of hearings which were intended to determine MDMA's permanent status. The Schedule I status is restricted for those drugs which lack currently acceptable medical utility and have a high abuse potential. "DEA officials were reportedly surprised that a substantial number of people, including therapists and clergymen, supported a less restrictive categorization" (McDowell & Kleber, 1994, p. 128). Nevertheless, the drug was permanently placed in Schedule I. This was followed by the synthesis and distribution of 3,4-methylenedioxyethamphetamine, (MDE or Eve) a structural analog of MDMA. "Shortly after attempts to market Eve, Congress passed the Controlled Substances

Analog Act in 1986 (Designer Drug bill or CSAA) outlawing all new analogues of illicit drugs" (Beck & Rosenbaum, 1994). Designer drugs are variations of already federally regulated compounds which are designed to mimic the effects of the compounds from which they were derived. Prior to the enactment of the CSAA, chemists and drug dealers hastily synthesized analogs of already scheduled drugs thereby evading the Food and Drug Administration's list of restricted drugs. Hence, they were marketing an essentially legal drug. The hurried mass production of these analogs increased the risk of distributing a potentially hazardous compound since no systematically controlled experimental analysis occurred. The well-intentioned enactment of the CSAA did not eliminate the inevitable "grace period" between the synthesis and distribution of a novel analog and the federal control of that compound. Further, the abuse of these high-technology creations will likely continue, because such drugs are relatively inexpensive to produce and pharmacologically superior (more potent) than other illicit drugs (Carroll, 1993). Still, the behavioral and physiological effects of such compounds are virtually unknown preceding the distribution of them to many users. Generally, the effects of potentially hazardous compounds have occurred before the appropriate actions could be taken to inform users of such deleterious side-effects. "Without any quality control, designer drugs are often sold on the street contaminated with impurities and poisonous by-products" (Carroll, 1993, p. 21). For instance, the synthetic opiate 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been known to cause substantial damage to the substantia nigra resulting in swift and severe Parkinsonian-like symptoms (Langston & Palfreman, 1995). Likewise, MDMA has also been purported to produce untoward physiological

effects. While there is a considerable amount of published reports regarding the physiological effects of MDMA, its exact mechanism of action are yet unknown. Thus, further examination of the effects of MDMA is a noteworthy pursuit.

### The Neurochemical and Physiological Effects of MDMA

Piercey et al (1990) examined the effect of MDMA on neuronal firing rates and reported that high doses of MDMA potently depressed the firing rates of a subpopulation of serotonin neurons in the dorsal and median raphe and that dopamine neurons were unaffected, suggesting that MDMA's distinct effects are mediated through a subpopulation of serotonergic neurons. Neurochemical investigations have demonstrated that MDMA and its demethylated metabolite, MDA induce the presynaptic release of serotonin (5-HT) and dopamine (DA) (Johnson et al., 1986; McKenna et al., 1991; Yamamoto & Spanos, 1988). Several analyses of the individual isomers of these compounds have revealed that the (+)-isomers are more potent DA releasers than the (-)-isomers (Hiramatsu & Cho, 1990; Johnson et al., 1986; McKenna et al., 1991) and the (-)-isomers bind to 5-HT<sub>2</sub> receptors with higher affinity than the (+)-isomers (Lyon et al., 1986). Behavioral studies also indicate that (+)-MDMA is more potent than (-)-MDMA in disrupting operant responses (Rosecrans and Glennon, 1987) and causing stereotypy in rats (Hiramatsu et al., 1989).

The neurochemical, behavioral and physiological evidence indicate that the (+)-isomers of MDA and MDMA are more closely related to amphetamine, a potent DA releaser that also produces stereotypy, at sufficient doses, in rats. Further, the

stereoisomers of MDA and MDMA have been demonstrated to differ in the extent to which they produce stimulus generalization in subjects trained to discriminate between either amphetamine or a hallucinogen and a vehicle. For instance, (+)-MDA substitutes for amphetamine (Glennon & Young, 1984) and (-)-MDA substitutes for the hallucinogens 2,5-dimethoxy-4-methylphenylisopropylamine (DOM) (Glennon et al., 1982) and *α*-lysergic acid diethylamide (LSD) (Callahan & Appel, 1988). Although neither isomer of MDMA was found to substitute for DOM (Glennon et al., 1982) or LSD (Callahan & Appel, 1988), it has been reported (Glennon et al., 1988) that (+)-MDMA substitutes for amphetamine but (-)-MDMA does not. On the other hand, Oberlender and Nichols (1988) found that neither isomer of MDMA substitutes for amphetamine. Broadbent et al. (1992) reported that only (-)-MDA substitutes for LSD. In subjects trained to discriminate the individual isomers of MDA or MDMA (Baker et al., 1995; Broadbent et al., 1992), mescaline does not produce stimulus generalization while LSD substitutes for (-)-MDMA and both isomers of MDA. Such inconsistencies among the reported results seem to indicate that the extent to which the discriminative stimulus effects of the optical isomers of both MDMA and MDA are amphetamine- or hallucinogen-like depends on the training drug and the discrimination procedures utilized.

### Purpose of the Present Study

This procedure is a modification of the traditional two-lever drug discrimination assay which provides an assessment of the subjective, interoceptive stimulus properties of a range of drugs. Subjects are trained to discriminate one interoceptive stimulus

(drug) from another (vehicle). This paradigm is useful in the classification of novel compounds. It can be used to provide indirect information regarding underlying neurochemical events. In order to further delineate the discriminative stimulus properties of the stereoisomers of both MDA and MDMA, a three-choice drug discrimination procedure was employed. Employing a three-choice drug discrimination procedure in which subjects are trained to discriminate between two distinct drugs may further delineate the discriminative stimulus properties of the stereoisomers of MDA and MDMA.

## CHAPTER II

### METHODS

#### Subjects

Eight male Sprague-Dawley rats, maintained at 85% of their free-feeding weights, were used as subjects. The subjects were approximately eight months old at the beginning of the study. The subjects were exposed to operant conditioning procedures as part of an undergraduate learning lab prior to the initiation of the present study. The subjects were individually housed in hanging wire mesh cages. Water was provided ad libitum. The subjects were housed in a room with controlled lighting (12-hr light/12-hr dark) and temperature (20-22° C).

#### Apparatus

Eight operant conditioning chambers (Med Associates, East Fairfield, VT) measuring 28 cm long, 21 cm wide, and 21 cm high, were used. The front (21 X 21 cm) wall of each chamber was equipped with three response levers centered horizontally 7 cm above the floor. A dipper through which 0.1 ml sweetened condensed milk (diluted with tap water at a 1:2 ratio) was delivered was centered 5 cm below the levers. An exhaust fan provided masking noise and ventilation. The minimum force requirement for operation of a lever was 14 g. The top of the operant chambers were constructed of clear

plexiglass and the work panel and back wall were made of aluminum. Ambient illumination was supplied by a 7-watt light (housetlight) centrally located on the top of the work panel. Control of the experimental events and data recording was accomplished through the use of a Zenith Z-320/SX microcomputer (IBM compatible) using software and an interface designed by Med Associates (East Fairfield, VT).

### Drugs

LSD, DOM, d-amphetamine as well as the stereoisomers of MDMA and MDA were obtained from the National Institute on Drug Abuse (Rockville, MD). The doses were expressed as the salt. The drugs were dissolved in 85% physiological saline and were administered by intraperitoneal injection (i.p.).

### Training

The subjects were trained to discriminate amphetamine (1.0 mg/kg) and LSD (.08 mg/kg) from saline in a three-choice drug discrimination procedure under a fixed-ratio 20 (FR 20) schedule of reinforcement. All eight subjects were reinforced for responses on the center lever following saline injections. Four of the subjects were reinforced for responses on the right lever following injections of amphetamine and on the left lever following injections of LSD. The conditions were reversed for the remaining subjects. The drug and saline injections were administered 15 min. prior to 20 min. training sessions. The training sessions were conducted six days per week (Mon.-Sat.).

Training began under a FR 1 schedule. When responding was consistent and

stable, the FR was incremented gradually from 1 to 20. Reinforcement was contingent upon 20 consecutive responses on the correct lever. Incorrect responses on either lever reset the response counter. A semi-random schedule of drug administration was employed in order to ensure equitable presentation of all conditions. The percent of correct lever choice prior to the first reinforcer of each training session was used to determine discrimination acquisition. This ensured that responding was stimulus controlled and not contingency controlled.

### Testing

When each subject achieved a mean of at least 85% correct lever choice over a period of 10 consecutive training sessions, substitution tests began. Dose response determinations were generated for the training drugs amphetamine (.25, .5, 1.0, 2.0 mg/kg) and LSD (.02, .04, .08, .16 mg/kg). Vehicle control tests were also determined. (+)-MDA, (-)-MDA, (+)-MDMA (.31, .63, 1.25 mg/kg) and (-)-MDMA (.88, 1.75, 3.5 mg/kg) were tested for substitution. These doses were chosen on the basis of previous studies (Baker et al., 1995; Broadbent et al., 1992) in which subjects were trained to discriminate each of the isomers. DOM (.5, 1.0, 1.5 mg/kg) and cocaine (1.25, 2.5, 5.0, 10.0, 15.0 mg/kg) were also tested for substitution. The order of dose presentation was counter balanced within and between subjects. The substitution tests were conducted under extinction and concluded once 20 consecutive responses were emitted on either lever or 20 minutes elapsed, whichever occurred first.



## CHAPTER III

### RESULTS

For each dose tested, the mean percent of total responses on each lever was calculated and plotted for visual analysis. Complete generalization was defined as a mean of at least 80% of the total responses on any particular lever. Partial generalization was defined as a mean between 50% and 80% of the total responses on any particular lever. A two-factor repeated measures analysis of variance (ANOVA) was conducted for the LSD and amphetamine levers during substitution tests with the optical isomers of MDA and MDMA. The two factors were isomer and dose (3 dose levels and vehicle control). For substitution tests with LSD, DOM, amphetamine and cocaine, the percent of total responses on either the amphetamine or LSD lever was analyzed using a one-factor repeated measure ANOVA. Response rate (responses per second) during substitution tests was also calculated, plotted and statistically analyzed using a one-factor repeated measures ANOVA.

Each of the eight subjects' behavior came under stimulus control of all three drug conditions. The discrimination criterion (individual means of at least 85% correct lever choice prior to the delivery of the first reinforcer over 10 consecutive training sessions) was met in all eight subjects within an average of 100 training sessions.

Each of the eight subjects completed substitution tests with three doses of the

optical isomers of MDA and MDMA. Further, each subject completed dose response tests with the two training drugs. Vehicle control tests were completed by each of the subjects. Since the order of the substitution tests was random, the condition received prior to any particular test session was not the same for each subject. When LSD control tests were conducted, the condition each subject received during the previous session appeared to modulate the obtained results. For instance, the two subjects for which LSD control tests followed LSD training sessions, the mean percent of LSD-appropriate responses was much lower than the subjects which received amphetamine or saline during the preceding session. Each of these two subjects emitted 100% of their responses on the saline-appropriate lever. To eliminate acute tolerance as a variable, LSD control tests were conducted following each of the three conditions. The resultant control values plotted are a mean of all three tests. The group mean was 92% following amphetamine training sessions ( $N=8$ ). Following saline sessions, the mean was 76% ( $N=8$ ). The mean was only 53% following LSD training sessions ( $N=7$ ). Figure 1 shows the dose response curves for the training drugs. LSD produced dose dependent increases in LSD-appropriate responding (Figure 1, Graph A). The training dose produced 81% LSD-appropriate responding. LSD (.16 mg/kg) was also tested for substitution and it produced 60% LSD-appropriate responding. A one factor repeated measures ANOVA on percent LSD-lever responding revealed a significant dose effect ( $F_{4,35} = 4.74$ ,  $p < .05$ ). A one factor repeated measures ANOVA on LSD response rate revealed a nonsignificant dose effect ( $F_{3,21} = .73$ ,  $p > .05$ ). Figure 1 also depicts the dose response curve for amphetamine (Figure 1, Graph B).

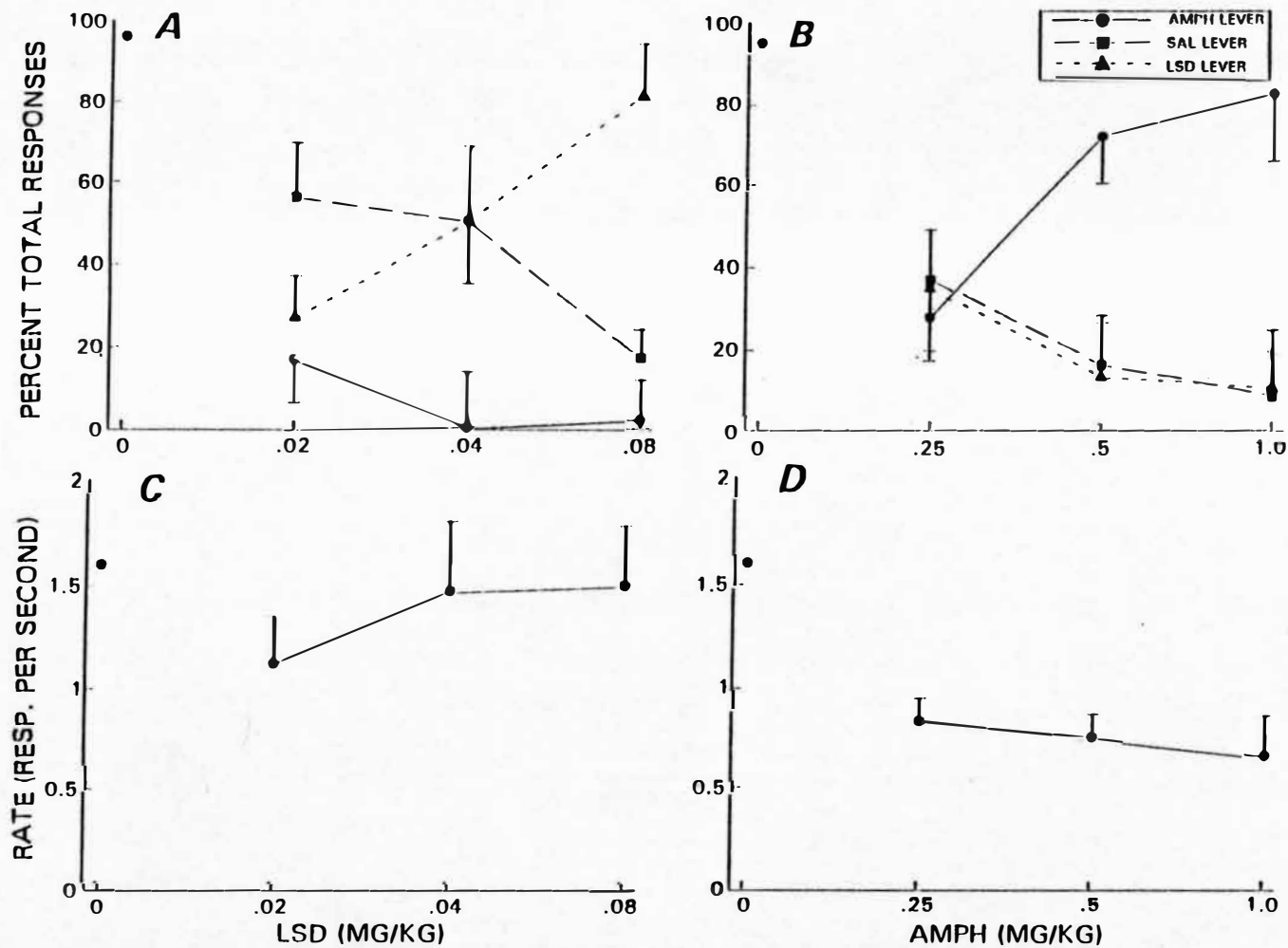


Figure 1. Dose Response Functions for the Training Drugs LSD (A & C) and Amphetamine (B & D) (N=8 at All Doses of Amphetamine and LSD).

A mean of 82% was obtained when the training dose of amphetamine (1.0 mg/kg) was tested. Amphetamine (2.0 mg/kg) (not shown) was also tested but diminished responding greatly. Most of the subjects did not complete the FR20 requirement. A one-factor repeated measures ANOVA on percent amphetamine-lever responding revealed a significant effect of dose ( $F_{3,21} = 20.01, p < .01$ ). Figure 1 (Graph D) depicts a significant decrease in response rate during amphetamine tests compared to saline control ( $F_{3,21} = 7.98, p < .01$ ).

To affirm that the subjective effects of the two distinct drugs had been established as discriminative stimuli, DOM and cocaine were tested for stimulus generalization. Figure 2 depicts the dose response functions for both DOM and cocaine. Figure 2 (Graph A) shows percent total responses for DOM. Visual analysis of the graph reveals that DOM produced dose dependent increases in LSD-appropriate responding. An overall mean of 87% was obtained at the highest dose tested. DOM was tested in seven of the eight subjects. A one-factor repeated measures ANOVA revealed a significant dose effect ( $F_{3,18} = 9.8, p < .01$ ) but a nonsignificant effect on rate ( $F_{2,12} = .20, p > .10$ ).

Figure 2 (Graph B) shows overall percent for cocaine. Initially, cocaine (2.5, 5.0 and 10.0 mg/kg) was tested for substitution. Since little variation in amphetamine-appropriate responding occurred (81%, 80%, and 83% respectively) cocaine 1.25 and 15.0 mg/kg) was also tested. All of the subjects ( $N = 5$ ) emitted at least 90% of their responses on the amphetamine lever at the highest dose tested (overall mean 96%). A one-factor repeated measures ANOVA revealed a significant dose effect ( $F_{5,20} = 9.0, p < .01$ ) as well as a significant effect on rate ( $F_{4,16} = 2.4, p < .05$ ).

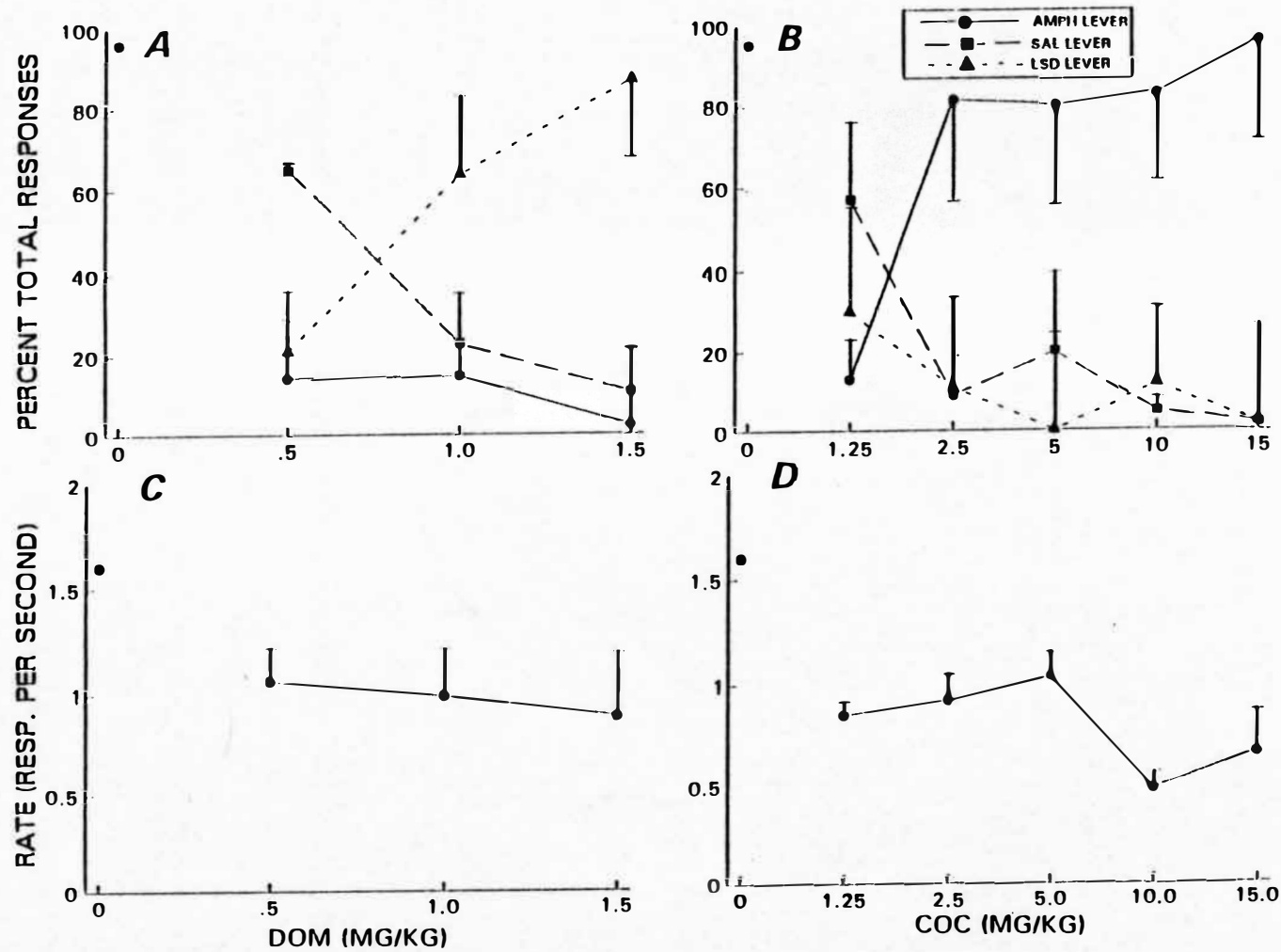


Figure 2. Dose Response Functions for DOM (N = 7) and Cocaine (N = 5).

Figures 3 and 4 show the dose response curves for the stereoisomers of MDA and MDMA. Visual inspection of the dose effect curves reveals that the optical isomers of both MDA and MDMA do not substitute for amphetamine. In fact, both (+)- and (-)-MDMA and (-)-MDA produced virtually no responding on the amphetamine lever. (+)-MDA (.63 mg/kg) engendered the greatest degree of amphetamine lever responding. Two of the eight subjects emitted 100% of their responses on the amphetamine-appropriate lever, while three others emitted 100% of their responses on the LSD-appropriate lever. The remaining subjects emitted 100% of their responses on the saline lever. (+)-MDA (.63 mg/kg) produced partial generalization of the subjects responding to the LSD-appropriate lever (66%). In contrast, all of the subjects allocated less than 5% of their responses on the amphetamine lever with (+)- and (-)-MDMA and (-)-MDA. While (+)-MDMA (1.25 mg/kg) produced the greatest amount of LSD-appropriate responding (71% of the responses occurred on the LSD lever). (-)-MDMA (1.25 mg/kg) produced nearly equal responding on the saline- and LSD-appropriate levers while the subjects emitted less than 2% of their overall responding on the amphetamine lever. Further, (-)-MDMA produced dose-dependent increases in LSD lever responses. (-)-MDMA also produced equitable responding on both the LSD and saline levers while all of the subjects emitted less than 2% of their responses on the amphetamine-appropriate lever.

A two-factor repeated measures ANOVA on percent LSD-lever responses following (+)- and (-)-MDMA tests revealed a significant dose effect ( $F_{3,56} = 5.9$ ,  $p < .01$ ) but no effect of isomers ( $F_{3,56} = 1.4$ ,  $p > .05$ ) compared to saline control

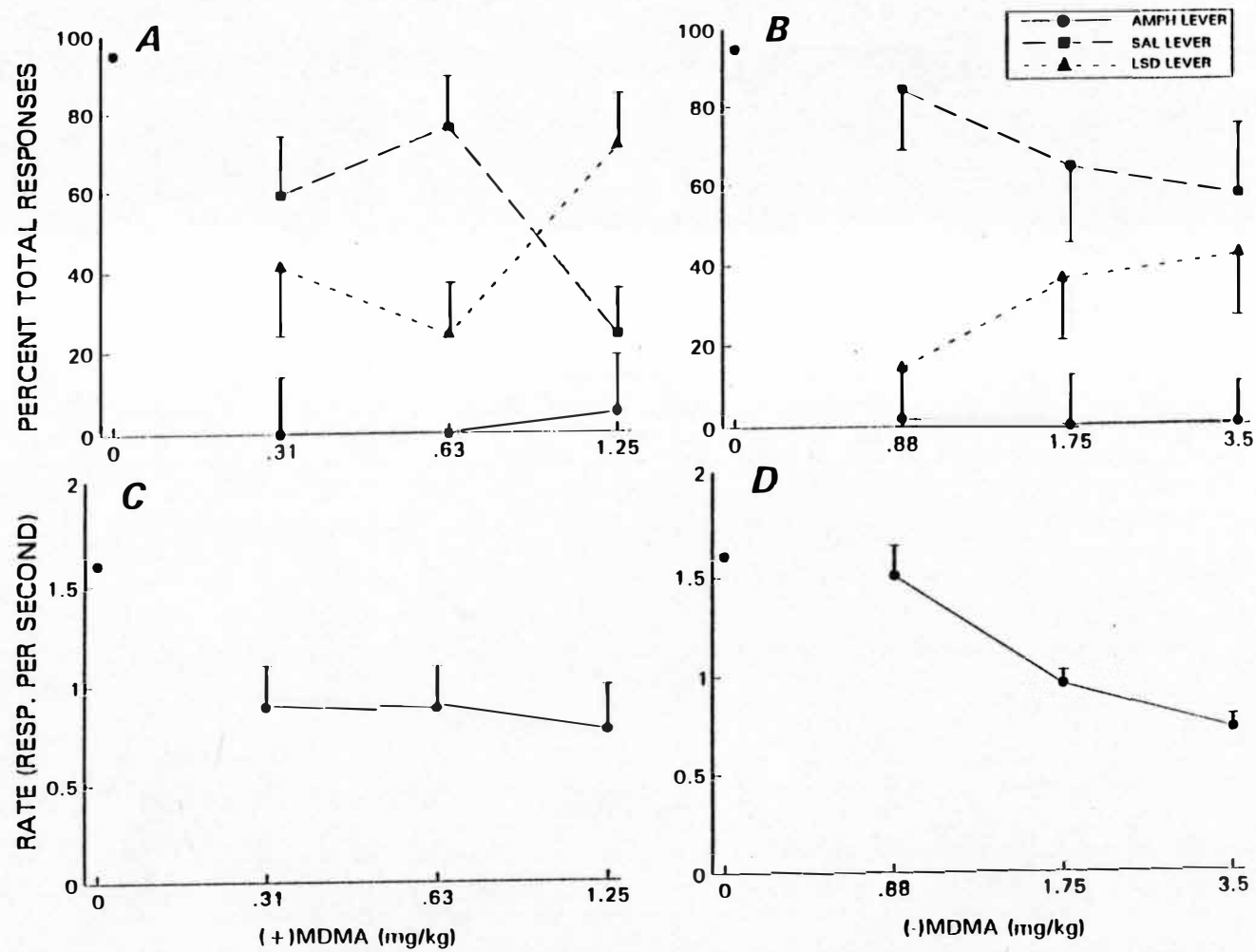


Figure 3. Dose Response Functions for (+)-MDMA and (-)-MDMA.

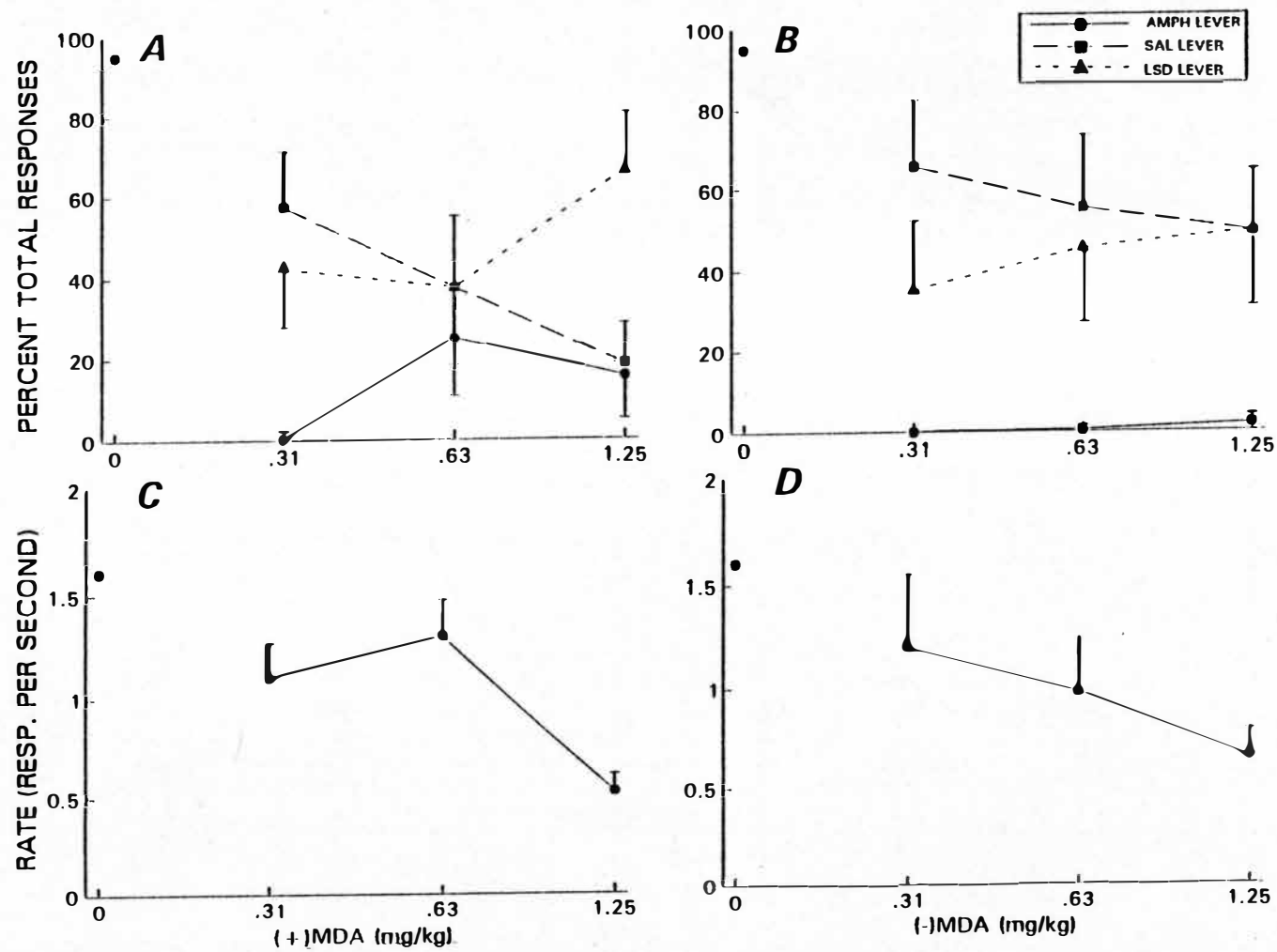


Figure 4. Dose Response Functions for (+)-MDA and (-)-MDA.



values. A significant dose effect on response rate following (-)-MDMA was obtained ( $F_{2,14} = 9.4$ ,  $p < .01$ ). A two-factor repeated measures ANOVA on percent LSD-lever and amphetamine-lever responses following (+)- and (-)-MDA tests was also conducted. There was not a significant effect of dose or isomer. However, there was a significant dose effect on response rate following (+)-MDA compared to vehicle control values. In addition, the results of the ANOVA revealed a nonsignificant dose and isomer effect on amphetamine-lever responding.

Since the optical isomers of both MDA and MDMA produced only partial generalization to LSD, one higher dose was tested for each isomer of both drugs. (+)-MDA, (-)-MDA, (+)-MDMA (2.5 mg/kg) and (-)-MDMA (5.0 mg/kg) were tested. (-)-MDMA (5.0 mg/kg) was behaviorally disruptive. The subjects laid flat on their stomachs and emitted very few responses. (+)-MDMA (2.5 mg/kg) also disrupted behavior. Another noted indication at this dose was clear fluid dripping from the mouth and nose. Such symptomatology implicates the 5-HT system. (+)-MDA (2.5 mg/kg) produced similar behaviors and symptoms. Conversely, (-)-MDA (2.5 mg/kg) did not disrupt responding and partially substituted for LSD. The obtained mean was 78%.

## CHAPTER IV

### DISCUSSION

The present study clearly demonstrates that rats can be trained to differentially respond to the subjective effects of both a hallucinogen and a stimulant in a three-choice drug discrimination assay. Thus, both amphetamine and LSD were serving as discriminative stimuli which controlled each subject's responding. The number of training sessions required to reach the discrimination criteria was approximately four times longer than is generally required in the traditional two-lever drug discrimination procedures employing either LSD (Callahan & Appel, 1988) or amphetamine (Glennon & Young, 1984). The results of the present study suggest that the discriminative stimulus properties of the stereoisomers of both MDA and MDMA are dissimilar to those of amphetamine. This is inconsistent with previous reports in which (+)-MDA and (+)-MDMA substitute for amphetamine (Glennon & Young, 1984; Glennon et al., 1988). Conversely, in subjects trained to discriminate between the optical isomers of MDA (Broadbent et., 1992) or MDMA (Baker et al., 1995) from saline, amphetamine did not completely substitute for any of these compounds.

Further, the results suggest that the optical isomers of MDA and MDMA are more similar to LSD than to amphetamine. The observation that the (+)-isomers of both MDA and MDMA produced a considerable amount of LSD-appropriate

responding is inconsistent with neuropharmacological evidence which suggests that the (-)-isomers have a higher affinity for 5-HT<sub>2</sub> receptors than the (+)-isomers (Lyon et al., 1986). However, (-)-MDA produced nearly complete substitution for LSD at the highest dose tested. This is congruent with a previous report which found that LSD substitutes for (-)-MDA (Broadbent et al., 1995).

These findings also confirm previous results obtained in our laboratory in which the stereoisomers of MDA and MDMA produced partial substitution for mescaline while very little amphetamine-lever responding occurred in subjects trained to discriminate between the two drugs and saline (Baker & Taylor, under review). However, these results are incongruent with those of Young and Glennon (1993). They demonstrated that rats can be trained to discriminate the optical isomers of MDA. They reported that amphetamine produced (+)-MDA-appropriate responding while DOM produced (-)-MDA-appropriate responding. In the present study, there was no evidence to support the notion of isomeric specificity. In other words, the (+)-isomers were not more similar to amphetamine than the (-)-isomers. Conversely, the stereoisomers of both drugs were more like LSD and all of the isomers produced negligible amounts of amphetamine-lever responding. The stereoisomers of MDMA and MDA produced only partial generalization of responding to the LSD-appropriate lever and did not produce a greater percentage of such responding following the administration of the high doses of any of the isomers. The highest dose of (+)-MDMA, (-)-MDMA and (+)-MDA was behaviorally disruptive and subjects emitted only one or two responses. Curiously, (-)-MDA (2.5 mg/kg) was not disruptive and produced

nearly complete generalization to the LSD-appropriate lever.

In contrast to previous reports from investigations in which subjects are trained to discriminate between amphetamine and saline (Glennon & Young, 1984; Glennon et al., 1988), the (+)-isomers do not appear to be amphetamine-like when subjects are trained to discriminate a hallucinogen as well. Clearly, the extent to which the stereo-isomers are hallucinogen- or stimulant-like appears to depend on the training drugs employed. Further, it appears that when subjects are trained to discriminate multiple drug stimuli (e.g., with LSD and amphetamine component), drugs which are tested for substitution that have similar components will not produce complete generalization to either component. The present findings tend to support the human subjective reports that MDMA and MDA produce subjective interoceptive stimuli that are similar to both stimulants and hallucinogens but are distinct from both of these traditional drug classes and may represent a novel therapeutic drug class named the entactogens (Nichols et al., 1986). A three choice drug discrimination assay may provide a greater degree of precision in which to investigate the complex discriminative stimulus properties of compounds than the more traditional two choice procedure.

**Appendix A**

**Western Michigan University Institutional  
Animal Care and Use Committee  
(IACUC)**

IACUC Number 94-07-02  
 Date of Receipt 3/7/94  
 Date of Approval 3-14-94

**WESTERN MICHIGAN UNIVERSITY  
 INSTITUTIONAL ANIMAL CARE  
 AND USE COMMITTEE (IACUC)**

Application to use Vertebrate Animals for Research or Teaching

The use of any vertebrate animals in research and/or teaching without prior approval of the Institutional Animal Care and Use Committee (IACUC) is a violation of Western Michigan University policies and procedures. This Committee is charged with the institutional responsibility for assuring the appropriate care and treatment of vertebrate animals.

Mail 6 copies of the typed application and any supplements to Research and Sponsored Programs, Room A-221 Ellsworth Hall, (616) 387-3670.

Any application that includes use of hazardous materials, chemicals, radioisotopes or biohazards must be accompanied with SUPPLEMENT A.

Any application that includes survival surgery must be accompanied with SUPPLEMENT B.

Lisa E. Baker  
 Principal Investigator/Instructor

Psychology  
 Department

7-4484  
 Campus Phone

Responsible Faculty Member  
 (if PI not faculty member)

Department

Campus Phone

Title of Project/Course: Evaluation of MDMA and MDA stereoisomers in a three-choice drug discrimination among a stimulant, a hallucinogen and saline.

Check One: Teaching        Research ☒ Other       

I. ANIMAL USE CATEGORIES (check ONLY one category)

- A. ☒ Projects that involve little or no discomfort (including injections).
- B.        Projects that may result in some discomfort or pain, but of short duration. Anesthetics, analgesics or tranquilizers will be used.
- C.        Projects that may result in significant discomfort or pain. Anesthetics, analgesics, or tranquilizers will not be used.

## II. ANIMAL USE FACILITIES

Please indicate the building and room(s) where the animal(s) will be housed and cared for as well as the location of the experiments and procedures if different from where housed.

Animals will be housed and cared for in Wood Hall, room 289. The experimental procedures will be conducted in Wood Hall, room 227.

## III. ANIMAL USE SUMMARY

In language understandable to a layperson, summarize your primary aims and describe the proposed use of animals as concisely as possible. Bear in mind that the IACUC is primarily interested in the responsible, necessary, humane use of animals. Include a description of procedures designed to assure that discomfort and pain to animals will be minimized. It should include method of restraint; method of dosing with test compound; and methods of euthanasia or disposition of the animal after the experiment.

This study involves a series of experiments, the primary objective of which is to further explore the stimulus effects of MDMA and MDA stereoisomers. A brief rationale and description of the proposed experiments follow.

Some investigators have suggested that the individual stereoisomers of the designer drugs MDMA and MDA have qualitatively distinct stimulus effects. For example, (+) MDA is presumably more similar to the psychomotor stimulant amphetamine, while (-) MDA is more similar to hallucinogenic substances, in particular, DOM and mescaline (Glennon and Young, 1982; 1984). Results of our research (in particular, a series of experiments I conducted prior to coming to WMU) are not in complete agreement with this notion. The purpose of the proposed series of experiments is to further examine the stimulant and hallucinogenic components of the stereoisomers of MDMA and MDA. While the majority of drug discrimination studies with these compounds have involved two lever (drug vs saline) discriminations, the present experiments will involve training a 3 lever (drug vs saline vs drug) discrimination.

### Discrimination Training:

Male Sprague-Dawley rats will be trained to discriminate a psychomotor stimulant (d-amphetamine OR cocaine) from an hallucinogen (mescaline OR LSD) and from saline in a three-lever operant task. Sixteen animals will be trained to discriminate cocaine-saline-LSD and another sixteen animals will be trained to discriminate d-amphetamine-saline-mescaline. To establish these discriminations, rats will be administered an intraperitoneal injection of one of the training drugs or saline and placed in an operant chamber 15 min later. Reinforcers (0.1 ml sweetened condensed milk diluted 50% in water) will be delivered for responses on the correct lever, which will vary depending on which compound is administered. Twenty min training sessions will be conducted once per day, 6 days a week (Mon-Sat). Drug and saline training sessions will alternate on a random basis (e.g. SSAAASSSMMMSSASMSS..., S=saline, A=amphetamine, M=mescaline). During training, only one drug will be administered per day. Animals may receive a drug on an average of 4 days per week, but no animal will receive a drug for more than three consecutive days.

### Testing:

When animals have achieved the discrimination criterion (minimum 85% correct responding for 10 consecutive days), substitution tests will begin. These tests involve administering a novel compound in place of the training drug, and allowing the animal to complete 20 consecutive responses on either lever without reinforcement. Each of the following compounds will be tested over a wide range of doses: (+) MDA (0.125-1.5 mg/kg); (-) MDA (0.125-1.5 mg/kg); (+) MDMA (0.125-2.5 mg/kg); (-) MDMA (0.125-3.5 mg/kg). In addition, at least two lower doses of each of the training drugs will be tested. At least three training sessions will occur between substitution tests. After these tests have been completed, additional tests may be conducted in some animals with dopaminergic and serotonergic antagonists given

in combination with each training drug. The antagonists consist of haloperidol (0.125 - .5 mg/kg), Sch 39166 (0.1-1.0), and pirenpirone (0.02 - 0.32 mg/kg).

**Persons involved in animal training:**

Graduate and undergraduate students who have been (and will be) trained under my supervision will be running these experiments. Each student will run 16 animals per day. I will be responsible for overseeing that the experimental procedures are carried out properly and directing the order of test administrations. I will also be responsible for routine observation of each animal's health throughout the duration of these studies.

**Euthanasia:**

Following the completion of behavioral experiments, animals will be euthanized by CO<sub>2</sub>. Their brains may be removed and preserved for later histological procedures.

**References:**

- Baker et al. (1994) Discriminative stimulus effects of the stereoisomers of 3,4-Methylenedioxymethamphetamine (MDMA): involvement of serotonin release. submitted for review.
- Glennon, R. A., Young, R., Rosecrans, J. A. and Anderson, G. M. (1982). Discriminative stimulus properties of MDA analogs. *Biological Psychiatry*. 17: 807-814.
- Glennon R.A. and Young R. (1984). Further investigation of the discriminative stimulus properties of MDA. *Pharmacology, Biochemistry and Behavior*. 20: 501-505..



#### IV. JUSTIFICATION FOR ALL ANIMAL EXPERIMENTS

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Please provide a narrative with reference sources which addresses each of the following:

- A. What assurance can be provided to indicate that the procedure is not duplicative?  
I have done a fairly extensive review of the drug discrimination literature, and am fairly certain no one has conducted the experiments described in this protocol.
- B. Have non-live animal techniques (e.g. in vitro biological systems, computer simulation, audiovisual demonstration) been considered? Explain why they have not been utilized.  
My research questions involve behavioral measures of drug effects, which are not possible to obtain without live organisms.
- C. Why has this species been selected for this procedure?  
The principal investigator has extensive experience with this species in this experimental procedure.
- D. How many animals will be used in this project? How often will its procedures be done and over what duration?  
A minimum of thirty-two animals will be used for this study (n=16 for each training group). Procedures will be conducted six days a week. The estimated time to complete the study is about one year.
- E. In light of concern to minimize the number of animals used in experimentation, how will you determine the number of animals to be used?  
Sixteen animals will be trained on each of two three lever discriminations. A minimum of eight animals will be required to complete all test sessions in order to conduct appropriate statistical tests on the data. Since these may be difficult discriminations to learn, and since the duration of the study is fairly long, it is necessary to train at least twice the minimum required to complete all tests. This judgment is based on my experience with drug discrimination procedures.
- F. What is the anticipated pain or distress response of the animal; and what is the duration of discomfort? (Injections not included.)  
Minimal pain due to injections
- G. How will the pain in the animal be monitored?  
N.A.
- H. What sedative, analgesic, or anesthetics will be used, if any? Include dose, route and frequency of administration.  
N.A.
- I. What is the justification if pain relieving drugs are not used?  
N.A.

**WESTERN MICHIGAN UNIVERSITY  
INVESTIGATOR IACUC CERTIFICATE**

Title of Project: Evaluation of MDMA and MDA stereoisomers in a three-choice drug discrimination among a stimulant, a hallucinogen and saline.

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

  X   Approved

\_\_\_\_\_ Approved with the provisions listed below

Provisions or Explanations:

With euthanasia corroborate and/or assure death  
by opening the thoracic cavity.

Donald Geering  
IACUC Chairperson

3-14-94  
Date

Acceptance of Provisions

Alice E. Baker  
Signature: Principal Investigator/Instructor

3-22-94  
Date

J. Geering  
IACUC Chairperson Final Approval

3-23-94  
Date

#94-03-02

## BIBLIOGRAPHY

- Baker, L. E., Broadbent, J., Michael, E. K., Matthews, C. A., Methosh, R. B., West, W. B., & Appel, J. B. (1995). Assessment of the discriminative stimulus effects of the optical isomers of ecstasy (3,4-methylenedioxymethamphetamine; MDMA). Behavioral Pharmacology, 6, 263-275.
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