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A Three-Choice Drug Discrimination Procedure Differentiates the Stimulus Effects of d-Amphetamine and MDMA

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by

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(±)-3,4-Methylenedioxymethamphetamine (MDMA) produces effects in humans that are reportedly similar to those of CNS stimulants. However, drug discrimination studies in nonhumans have yielded inconsistent results regarding the similarities between MDMA and d-amphetamine. Sixteen male Sprague-Dawley rats were trained to discriminate d-amphetamine, MDMA, and saline in a three-lever drug discrimination procedure. In addition, differential outcomes were employed during drug discrimination training with eight of the rats but this did not appear to facilitate the acquisition of the discrimination. Cocaine (0.25-10.0 mg/kg) produced dose-dependent increases in d-amphetamine-appropriate responding with complete substitution at the highest dose administered. LSD (0.02-0.16 mg/kg), produced dose dependent increases in MDMA-appropriate responding and nearly complete substitution (78%) at the 0.08 mg/kg dose. Fenfluramine (1.0-4.0 mg/kg), (+)MDA (0.375-1.5 mg/kg), and (-)MDA (0.375 mg/kg), all produced dose-dependent increases in MDMA-appropriate responding. The serotonin antagonist Pirenperone (0.16-0.64 mg/kg) partially blocked the stimulus cue of MDMA.

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INTRODUCTION

The Drug Discrimination Procedure

Drug discrimination procedures are frequently used to classify the stimulus properties of psychoactive drugs. Stimulus control refers to "instrumental behavior under the control of particular stimuli that are present when the response is reinforced" (Domjan & Burkhard, 1993, p. 221). Stimuli used in the study of stimulus control can be divided into two categories: interoceptive (internal stimuli or private events such as pain) and exteroceptive (environmental stimuli such as tones or lights). In the drug discrimination procedure, drugs serve as internal discriminative stimuli, signaling when a particular behavior will be reinforced. That is, following repeated sessions where reinforcement has been available to the subject after administration of a drug, the detection of the presence of that drug serves as a signal to the subject that reinforcement is available. Generally, drug discrimination studies employ a two-choice operandum where subjects receive a psychoactive drug or vehicle (i.e., saline). In order to receive a reinforcer (e.g., water in a water deprived subject), subjects are required to perform one behavior in the presence of the psychoactive drug (e.g. press one lever) and a different behavior when the presence of drug is not detected (e.g., press a different lever).

D'Mello and Stolerman (1978) reported that, generally, all drugs of abuse and most psychoactive drugs demonstrate discriminative control. 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).

Once subjects have met the criterion for discrimination, other psychoactive drugs may be administered to examine if these other drugs possess similar discriminative stimulus properties to the training drug. This testing procedure is usually performed under extinction conditions. That is, subjects are removed from the operant chamber prior to the delivery of any reinforcement. Generally, if the resulting behavior is 80% or greater responses on a drug-appropriate lever during the testing sessions, then that substance is said to produce "substitution". "Substitution" refers to the idea that the novel substance produces generalization to one of the training conditions, indicating that it has similar stimulus properties. Additionally, an antagonist drug may be administered prior to the training drug to determine if such a compound will disrupt the stimulus control exhibited by the training drug. An antagonist is said to have "blocked" the effects of a training drug if the result is 80% or greater vehicle-appropriate responding. Antagonist testing is often used to study the neural mechanisms involved in producing the discriminative stimulus effects of psychoactive drugs.

However, there are limitations to consider when utilizing the drug discrimination paradigm. It is difficult to interpret results if testing procedures produce between 20% and 80% drug-appropriate responding, what is called "partial substitution". This result 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, 1987).

When subjects respond on the vehicle-appropriate lever after administration of a testing compound, one may not conclude the stimulus effects of this compound are necessarily opposite those of the training compound (Seiden & Dykstra, 1977), only distinctly different. Thus, regardless of the stimulus properties of a particular compound, the nature of the drug discrimination assay results in subjects responding regardless of the effects experienced. Researchers must be cautious in their interpretations of results obtained when using the drug discrimination assay, as it essentially measures the subjective effects of drugs.

Various methods have been employed in an attempt to compensate for these limitations. One such method is the utilization of more complex discriminations, such as three-choice discriminations, or drug versus drug discriminations (Stolerman, 1993, chap. 9). Moreover, despite the limitations, the drug discrimination assay serves as a useful tool to describe drugs as similar or dissimilar to training compounds, as well as to examine neural mechanisms involved in the effects of psychoactive drugs (Appel & Cunningham, 1986).

Drug Discrimination Investigations of MDMA and Amphetamine

The psychoactive drug (±)-3,4 methylenedioxymethamphetamine (MDMA) is a commonly abused drug reported to amplify self awareness, and promote empathy and communication (Solowij, Hall & Lee, 1992). MDMA shares both amphetamine

and (+)-lysergic acid diethylamide (LSD) properties although hallucinations are not often experienced by users (Downing, 1986). MDMA is a structural analog to d-amphetamine. However, human reports describe the subjective effects of MDMA as distinctly different from those of psychostimulants (Grinspoon & Bakalar, 1986; Solowij, Hall, & Lee, 1992). Those individuals surveyed reported MDMA primarily produces euphoria, an increase in feelings of intimacy and empathy, as well as intensified sensations and perceptions (Solowij et al., 1992). However, stimulant-like effects were also reported. These include, motor restlessness, tremors, ataxia, and sympathomimetic effects such as tachycardia and an increase in sweating (Solowij et al., 1992). It is likely that MDMA possesses complex stimulus properties that make it difficult to classify into the traditional drug classes (Baker & Taylor, 1997; Nichols, 1986; Nichols, Hoffman, Oberlander, Jacob, & Shulgin, 1987). Indeed, Nichols (1996) has proposed a separate class to characterize MDMA and similar amphetamines, for which he has coined the term "entactogens".

Investigations of the stimulus generalization between MDMA and d-amphetamine have yielded conflicting results. Glennon and Young (1982) reported that in rats trained to discriminate d-amphetamine from saline, (±)-MDMA produced stimulus generalization. The generalization of (±)-MDMA to d-amphetamine in pigeons was also reported by Evans and Johanson (1986). However, Oberlander and Nichols (1988) failed to replicate these findings in rats. In addition, at least two studies reported that animals trained to discriminate MDMA from saline do not generalize to d-amphetamine (Glennon & Misenheimer, 1989; Schecter, 1987).

Baker and Makhay (1994) reported partial substitution of amphetamine in MDMA trained rats. Interestingly, after administration of a neurotoxic dose regimen of fenfluramine, the highest dose of d-amphetamine tested (1.0 mg/kg) produced stimulus generalization. Moreover, Oberlander and Nichols (1988) reported that in animals trained to discriminate (±)-MDMA from saline, d-amphetamine did produce stimulus generalization but at a dose that severely suppressed responding in half of the subjects. Table 1 presents a synopsis of results from drug discrimination investigations of MDMA and d-amphetamine.

In an attempt to further characterize the discriminative stimulus effects of MDMA, Baker and Taylor (1997) utilized a three-choice discrimination procedure in which rats were trained to discriminate d-amphetamine and LSD from saline. They reported that neither isomer of MDMA substituted for d-amphetamine and actually produced more responding on the LSD-appropriate lever. These results indicate that MDMA produces discriminative stimulus effects that are distinctly different from those of d-amphetamine and may in fact be more similar to LSD. Moreover, it appears that the three-lever drug discrimination procedure is a more sensitive behavioral assay in which to investigate the discriminative stimulus effects of drugs with compound stimulus properties (Baker and Taylor, 1997). One of the aims of the present study was to determine whether rats could be trained to discriminate MDMA from d-amphetamine in a three-choice discrimination procedure.

The Differential Outcomes Effect

Goeters, Blakely, and Poling (1992) reported that differential outcomes shorten the time needed for acquisition of a response and also increase the terminal accuracy of the response. Differential outcomes refers to correlating each discriminative stimulus with a unique outcome. The efficacy of the differential outcomes effect is well established. Trapold (1970) first demonstrated a shortened time period for response acquisition and better accuracy in rats exposed to differential outcomes in a two-choice discrimination. Utilizing a standard two-lever operant chamber, a response on one lever was followed with one outcome (i.e., solid food) and a response on a different lever was followed by a different outcome (i.e., sucrose) (Trapold, 1970). Others have employed this assay with various experimental designs and numerous types of subjects (Goeters et al., 1992). Morgan and Baker (1997) demonstrated that differential outcomes increased the acquisition speed of the discrimination of cocaine from saline in rats. However, as Goeters et al. reported, the efficacy of the differential outcomes effect has not been thoroughly established when employed in experimental designs requiring subjects to respond to interoceptive discriminative stimuli (e.g., drug discrimination assay). Moreover, Goeters et al. reported that the usefulness of the differential outcomes effect appears to increase as the difficulty of the task increases. Thus, examination of the utility of the differential outcomes effect in a complex drug discrimination would be beneficial to researchers in this vast field.

Purpose of the Present Study

The primary goal of the present study was to determine whether rats could learn to discriminate the stimulus effects of both (±)-MDMA and d-amphetamine in a three-choice drug discrimination procedure, and to determine whether differential outcomes paired with each stimulus condition would facilitate the acquisition of this complex drug discrimination.

METHODS

Subjects

Sixteen experimentally naïve, male Sprague-Dawley rats served as the subjects. The subjects were individually housed in wire mesh cages, in a colony maintained on a 12-h light (0700 to 1900)/ 12-h dark cycle, at a consistent temperature of 20-22° C and at a constant humidity of 70-80%. Subjects had free access to standard laboratory rat chow. Access to water was restricted to 15-20 min following training and testing sessions and to a 24 hour period on weekends. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Western Michigan University and the subjects were maintained according to the general principles of animal husbandry outlined by the National Institutes of Health (see Appendix A).

Apparatus

Eight standard operant chambers (MED Associates Inc., St. Albans, VT, ENV-001) were used for all training and testing procedures. The operant chambers were located within sound and light attenuating shells with ventilation and masking noise. A 28 v house light was located in the front panel above the center lever. The dipper (0.1 ml) used to deliver the reinforcer was located below the center lever.

Drugs

The d-amphetamine sulfate; (±)-MDMA hydrochloride; cocaine hydrochloride; (+)-lysergic acid diethylamide (LSD); fenfluramine hydrocholoride; and both isomers of 3,4-methylenedioxyamphetamine hydrochloride (MDA) were obtained from the National Institute on Drug Abuse (Rockville, MD). Pirenpirone was purchased from Research Biochemicals International (Natick, MA). All drugs were dissolved in 0.9% bacteriostatic sodium chloride. Additionally, a few drops of acetic acid was added to dissolve the pirenperone in the saline vehicle.

Training Procedures

Subjects were trained to discriminate d-amphetamine (1.0 mg/kg) and (±)-MDMA (1.5 mg/kg) from saline in a three-lever drug discrimination under a fixed-ratio 10 (FR 10) schedule of liquid reinforcement. Injections were given intraperitoneally (IP), with a presession interval of 15 min.

An autoshaping procedure was utilized for the first week of training. Subject received between 5 and 6 sessions and no substance was administered prior to these sessions. Additionally, only the center lever was present in the operant chambers during this period of autoshaping. Following the autoshaping procedure, errorless discrimination was employed. That is, only the condition-appropriate lever was present for alternate training sessions of saline and each drug administration until each subject was exposed to at least four errorless discrimination training sessions

under each of these three conditions. At this point all three levers were introduced to each subject and were present for the remaining training sessions for the duration of the study. Training began with a FR 1 schedule of reinforcement for each condition and the ratio was gradually increased to 10 as responding became stable. 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. With administration of d-amphetamine, half of the subjects in both groups were reinforced for responses on the left lever and half were reinforced for responses on the right lever. Conditions were reversed for administration of (±)-MDMA. Under saline conditions, all subjects were reinforced for responses on the center lever. All levers were wiped with isopropyl alcohol between training sessions to reduce the effects of olfactory cues in the operant chambers (Extance and Goudie, 1981). Additionally, the order of groups was occasionally reversed for training sessions.

Eight of the sixteen rats were in the differential outcomes group where (±)-MDMA and d-amphetamine were correlated with either plain sweetened condensed milk or chocolate flavored sweetened condensed milk as reinforcers. Saline was correlated with water for all subjects in the differential outcomes group. The control group received water, plain and chocolate sweetened condensed milk on a random basis.

Saline, (±)-MDMA, and d-amphetamine were administered in a random order with subjects never receiving one condition for more than two consecutive sessions. Training sessions lasted for 20 min and were conducted six days a week at approximately the same time every day.

Testing Procedures

When subjects met the 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), stimulus generalization tests were administered with three different doses of each training drug (±) MDMA 0.375-1.5 mg/kg; d-amphetamine 0.25-1.0 mg/kg). Additionally, other test compounds (cocaine 1.0-10.0 mg/kg; LSD 0.02-0.16 mg/kg; fenfluramine 1.0-4.0 mg/kg; (+) MDA 0.375-1.5 mg/kg; and (-)MDA 0.375-3.0 mg/kg were tested for stimulus generalization. Stimulus generalization tests were conducted in a manner similar to training sessions, except no reinforcers were delivered and the animals were removed from the operant chambers immediately upon completion of 10 consecutive responses on any lever. Antagonist tests were also conducted. The pirenperone (0.16-0.64 mg/kg) was administered in conjunction with MDMA using a one hour preinjection period. That is, the pirenperone was administered one hour prior to the testing session, the MDMA was then administered fifteen minutes prior to the session.

Test sessions were conducted once or twice per week in place of training sessions provided the animals maintained 80% or better responding on each

condition-appropriate lever during training sessions. Following test sessions, animals received 20-30 min free access to water in their home cages.

Data Analysis

The number of sessions to criterion was calculated and a between-group comparison was made. Additionally, test data from the two groups (i.e., control and differential outcomes) were compared.

The mean percent of total responses on each lever for test sessions was calculated and displayed for visual analysis for each condition (i.e., (±)-MDMA, d-amphetamine, and saline). Rate was expressed as mean number of responses per second. Test data from animals that did not complete the FR 10 requirement were not included in the data analysis of testing sessions. For testing sessions, complete stimulus generalization was defined as at least 80% responding on either lever. For drugs that produced substitution, nonlinear regression analyses were calculated to determine ED_{50}s. Two way ANOVAs (group, dose) were conducted on each set of dose response data.

RESULTS

All sixteen subjects acquired the discrimination (minimum of 80% condition-appropriate responses prior to delivery of the first reinforcer in at least eight of ten consecutive training sessions) in the present experiment. Surprisingly, differential outcomes during training did not appear to facilitate acquisition of the discrimination. By 80 sessions, seven rats in the control group and six rats in the DO group had met the discrimination criterion. A t-test on the number of sessions to criterion at this point showed no significant difference between the two groups ($t = 0.11$, $df=11$, $p > 0.10$). The differential outcomes group met the discrimination criterion within 61 (SEM = 5.7, Range: 42-80, n=6) sessions, and the control group met this criterion within 60 (SEM = 2.9, Range: 53-74, n=7) sessions. Therefore, differential outcome training was discontinued. All sixteen subjects met the discrimination criterion before stimulus generalization testing began.

Figure 1 illustrates the results of stimulus generalization tests with MDMA. This training drug produced dose-dependent increases in the percentage of responses on the MDMA-appropriate lever and dose-dependent decreases in the percentage of responses on the saline-appropriate lever (ED_{50}=1.0 mg/kg). Very few responses were emitted on the d-amphetamine-appropriate lever during stimulus generalization tests with MDMA. A slight dose-dependent increase in response rate was observed. However, differences in response rate among doses were not statistically significant.

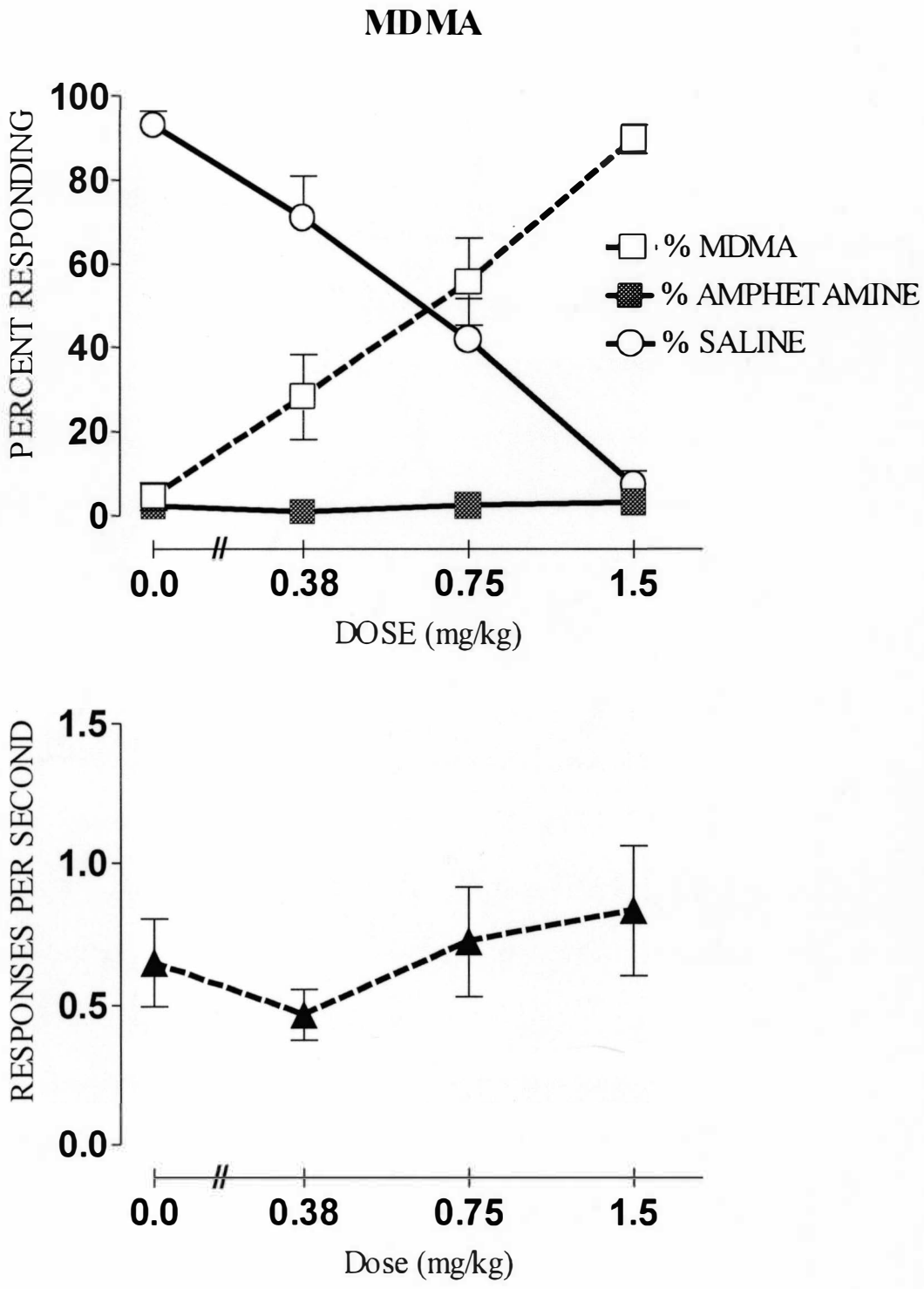

Figure 1. Results of MDMA Dose-Response Tests.

Additionally, a difference in percent MDMA-appropriate responding between the control group and the differential outcome group was observed ($F_{1,56}$=6.09, $p<0.05$). Figure 2 illustrates the MDMA dose-response curves for the differential outcome and control groups, and the response rate of both groups. There was no difference in response rates between groups. Two-way ANOVA's revealed no significant main effects of group on any of the other stimulus generalization tests.

The results of stimulus generalization tests with d-amphetamine are displayed in Figure 3. This training drug produced dose-dependent increases in the percentage of responses on the d-amphetamine-appropriate lever and dose-dependent decreases in the percentage of responses on the saline-appropriate lever (ED_{50}=0.33 mg/kg). At the training dose of d-amphetamine (1.0 mg/kg), virtually no responses were emitted on the MDMA-appropriate lever. It is interesting to note that the lowest dose of d-amphetamine (0.25 mg/kg), five of the 16 subjects emitted between 30 and 100% of their responses on the MDMA-appropriate lever. There was no statistically significant dose effect on response rate. However, there was a significant effect of group on response rate. That is, the control group responded at a higher rate then did the differential outcome group. ($F_{1,56}$=4.82, $p<.05$). Figure 4 illustrates the dose-response data and response rate data for the differential outcome and control groups. Statistical analysis resulted in no other significant differences between the control and differential outcome groups.

The administration of cocaine produced a dose-dependent increase in

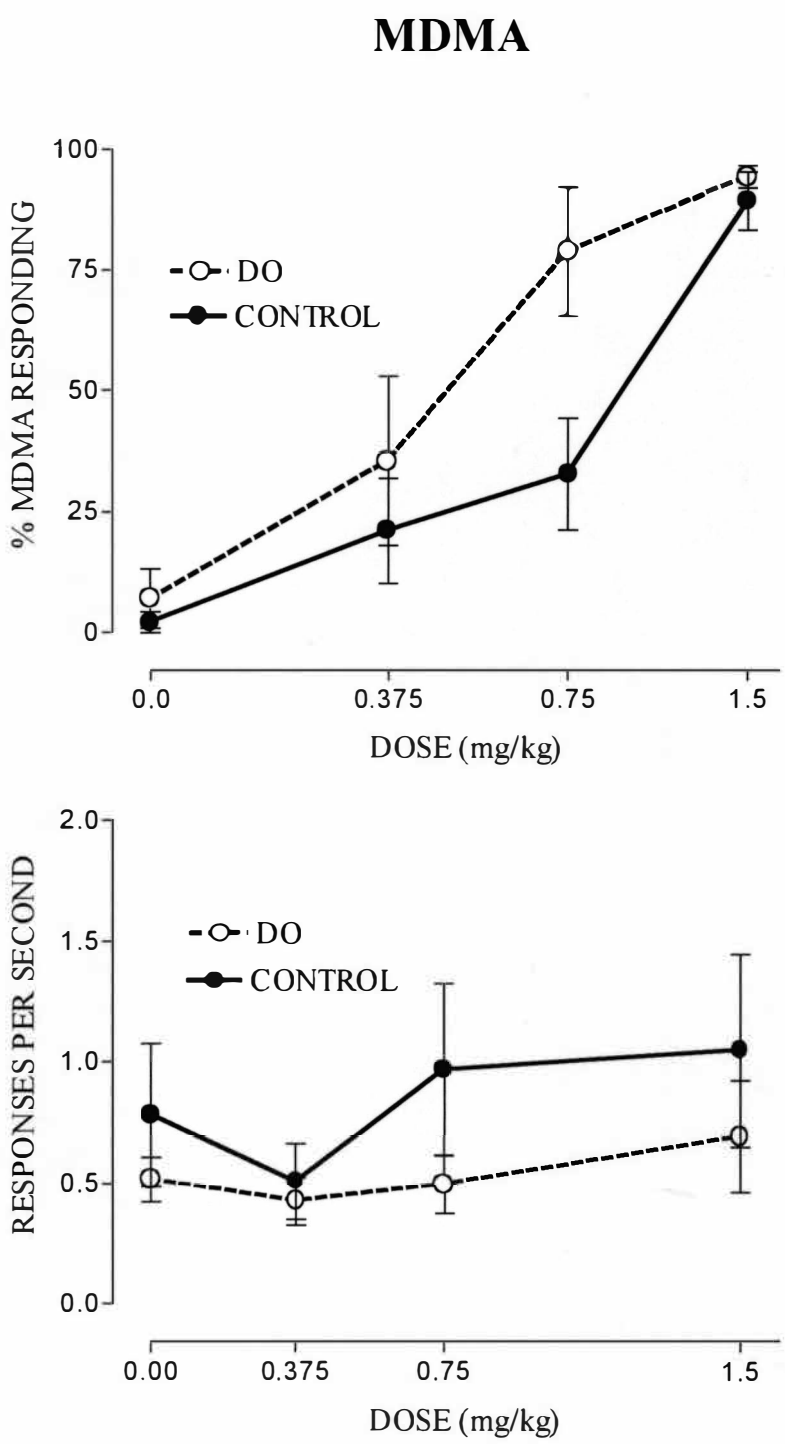

Figure 2. Results of MDMA Dose-Response Between Group Comparisons.

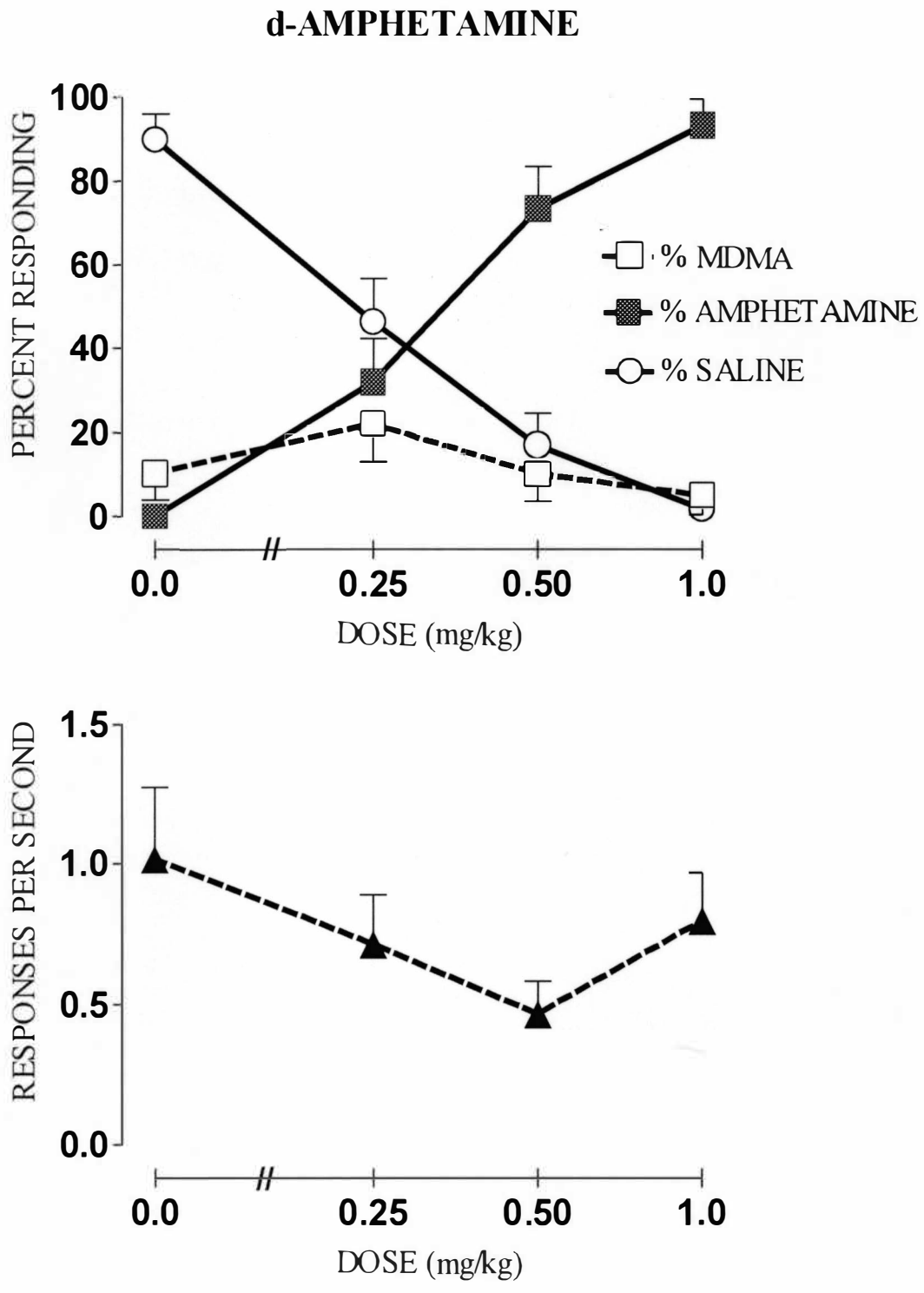

Figure 3. Results of d-Amphetamine Dose-Response Tests.

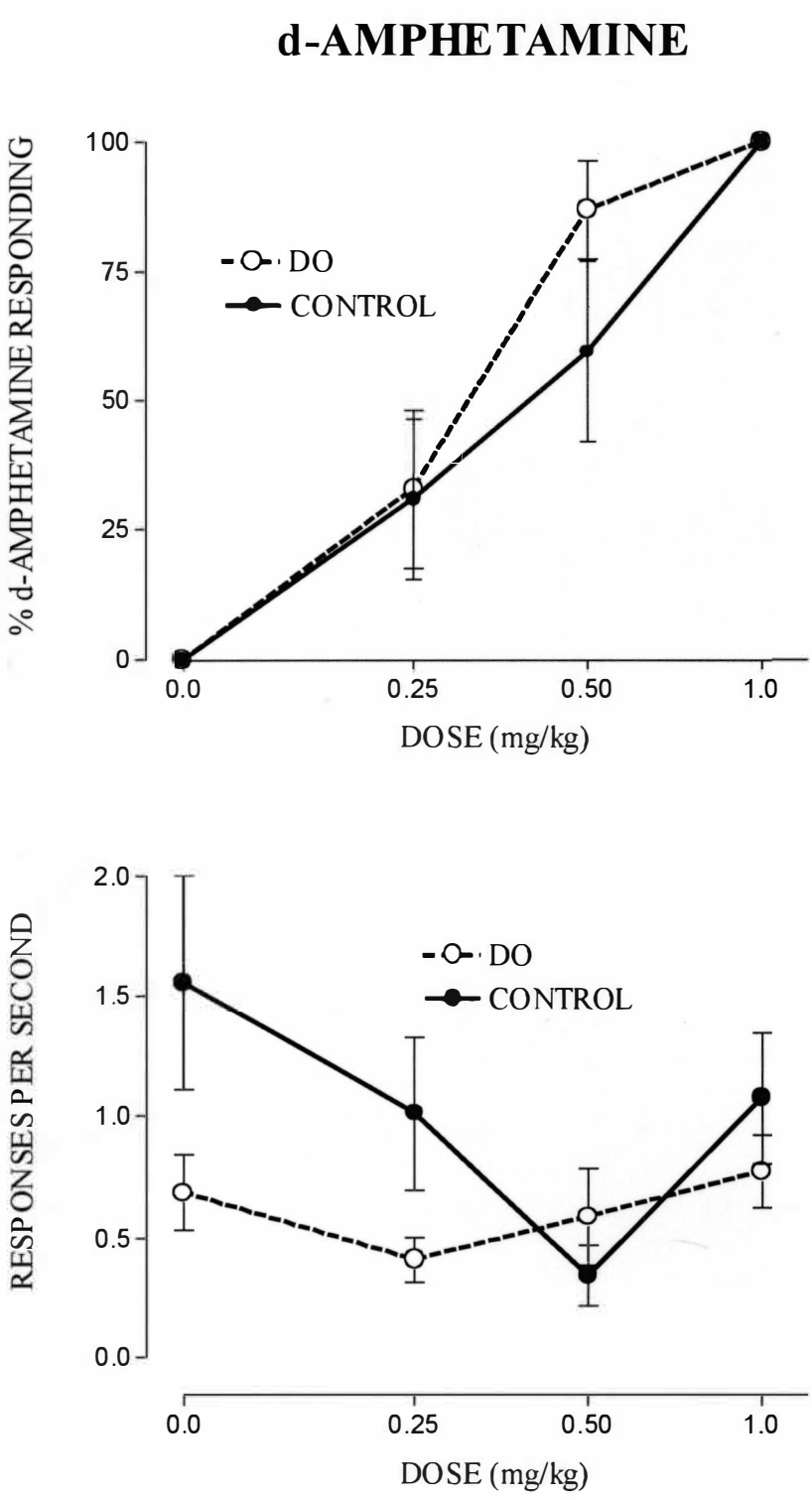

Figure 4. Results of d-Amphetamine Dose-Response Between Group Comparisons

d-amphetamine-lever responding with complete stimulus generalization at the highest dose (10 mg/kg; ED_{50}=3.12 mg/kg; see Figure 5). There was no significant difference in response rates across doses.

LSD produced nearly complete substitution (78%) for MDMA (see Figure 6). Of the 16 animals tested, 12 exhibited complete stimulus generalization at the 0.08 mg/kg dose, one exhibited partial generalization (58%) and three exhibited predominantly saline-appropriate responding (ED_{50}=0.04 mg/kg). Fourteen rats were tested at 0.16 mg/kg LSD, but this did not increase MDMA-appropriate responding. The highest rate of responding was seen at the lowest dose of LSD (0.02 mg/kg) with a dose-dependent decrease in responding.

The serotonin releaser fenfluramine produced nearly complete substitution for MDMA (see Figure 7) at a dose of 2.0 mg/kg. Eight of the 10 animals that tested at this dose exhibited complete generalization to MDMA (ED_{50}=0.56 mg/kg). Because of the high percentage of MDMA appropriate responding at the 2.0 mg/kg does, a dose of 4.0 mg/kg was administered. Response rate was severely depressed at this dose. Only two of the nine animals tested at the 4.0 mg/kg dose completed the 10 required response. Both of these subjects exhibited complete substitution for MDMA. Of the remaining seven animals, two subjects made six responses each, all of which were on the MDMA-appropriate lever. However, only the data from the two subjects that completed the test session were included in the data analysis.

Both isomers of MDA were also tested for stimulus generalization. (+)-MDA substituted completely for MDMA (see Figure 8) at a dose of 1.5 mg/kg (ED_{50}=0.70

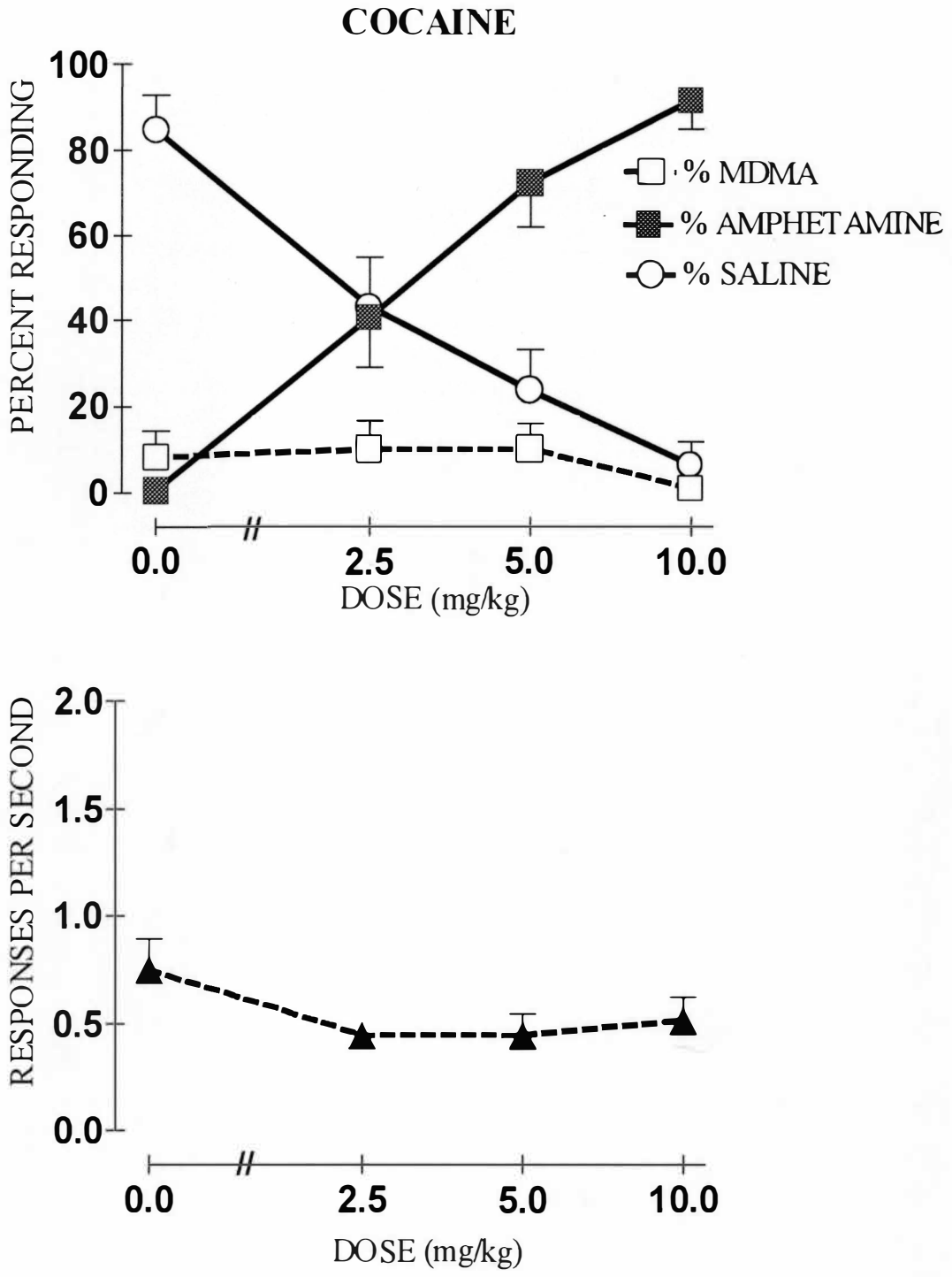

Figure 5. Results of Cocaine Dose-Response Tests.

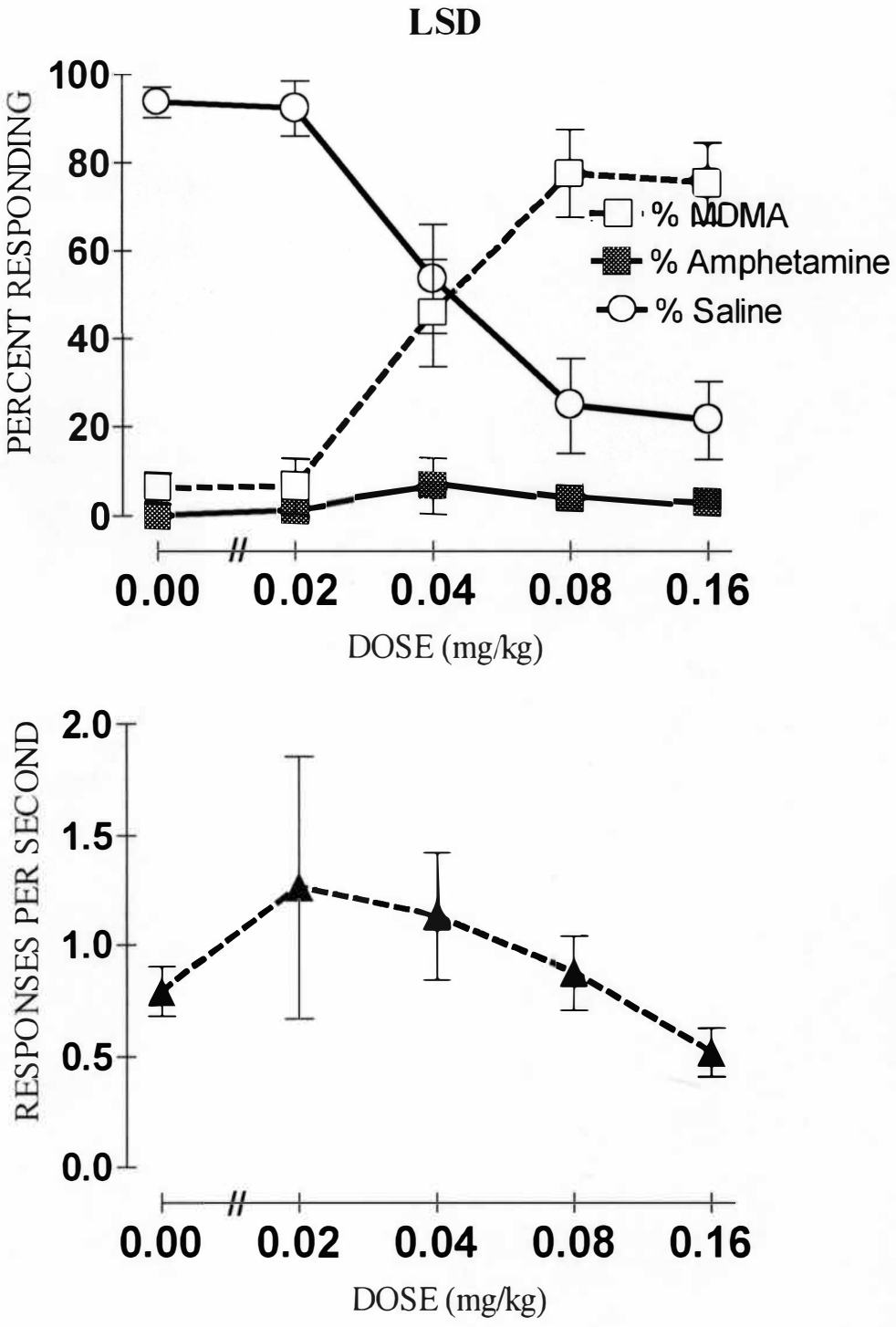

Figure 6. Results of LSD Dose-Response Tests.

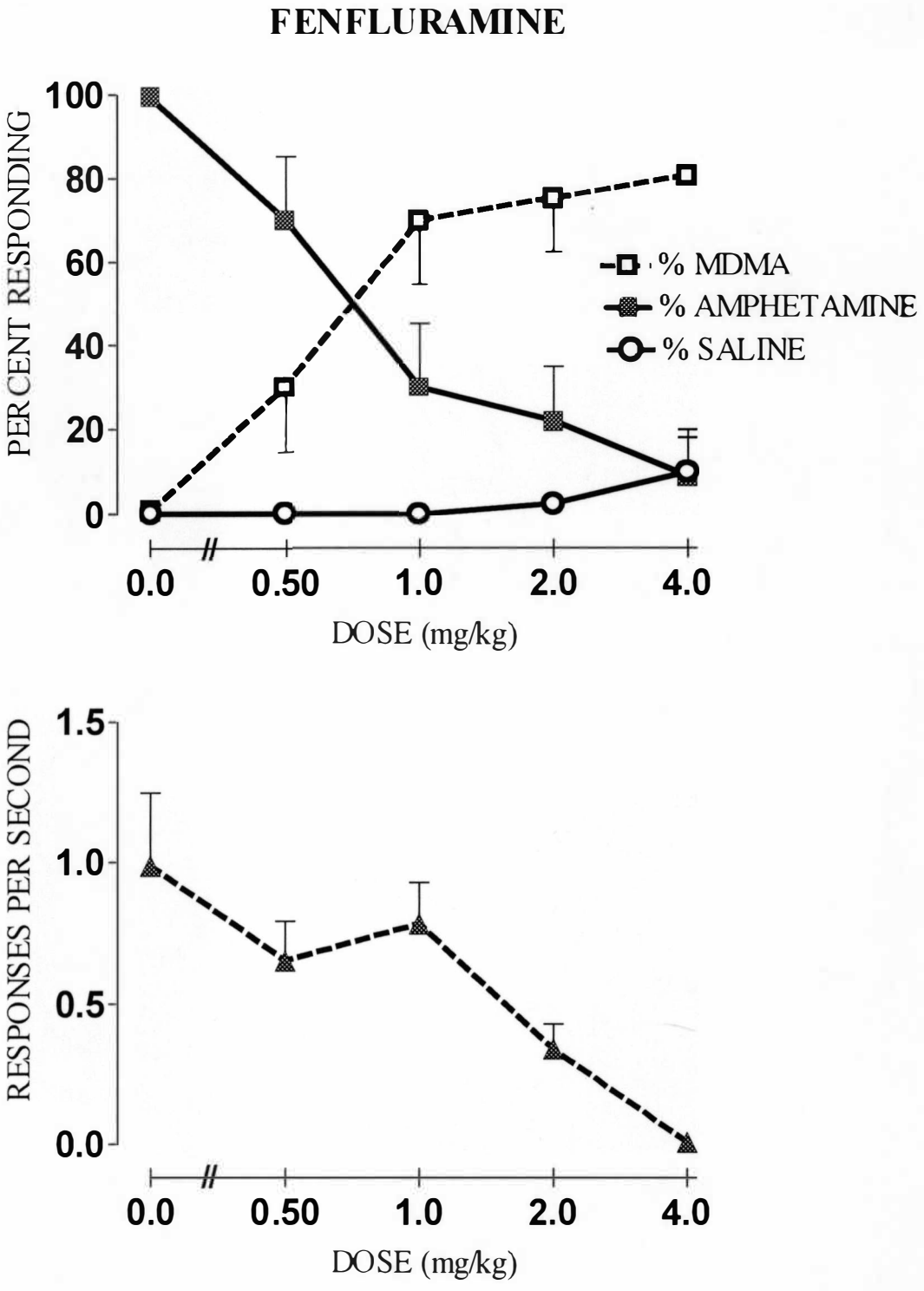

Figure 7. Results of Fenfluramine Dose-Response Tests.

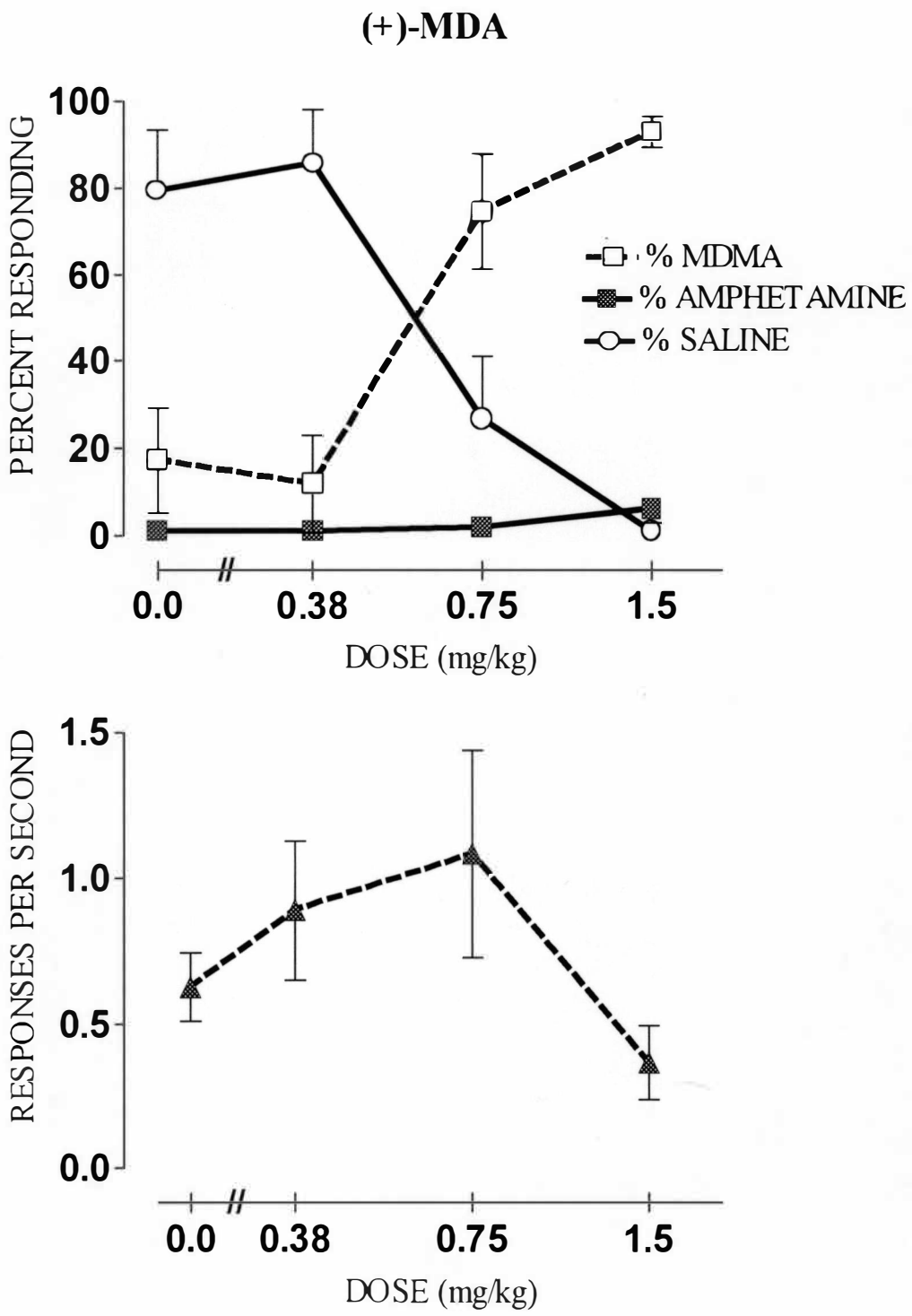

Figure 8. Results of (+)-MDA Dose-Response Tests.

mg/kg). Response rates decreased in a dose-dependent manner. The administration of (-)-MDA resulted in partial substitution at a dose of 1.5 mg/kg. A dose of 3.0 mg/kg was then administered to nine subjects and five of the subjects completed the required 10 responses. Complete stimulus generalization was exhibited by this dose in these animals (ED_{50}=2.17 mg/kg; see Figure 9).

Figure 10 illustrates the results of the administration of the serotonin antagonist pirenpirone in conjunction with MDMA. The result was a dose-dependent decrease in MDMA-appropriate responding. However, the highest dose tested (0.64 mg/kg) did not produce complete blockade (42 %) of the MDMA cue. The highest degree of blockade was observed with the 0.32 mg/kg dose (56%). Response rate differed very little across doses of pirenperone.

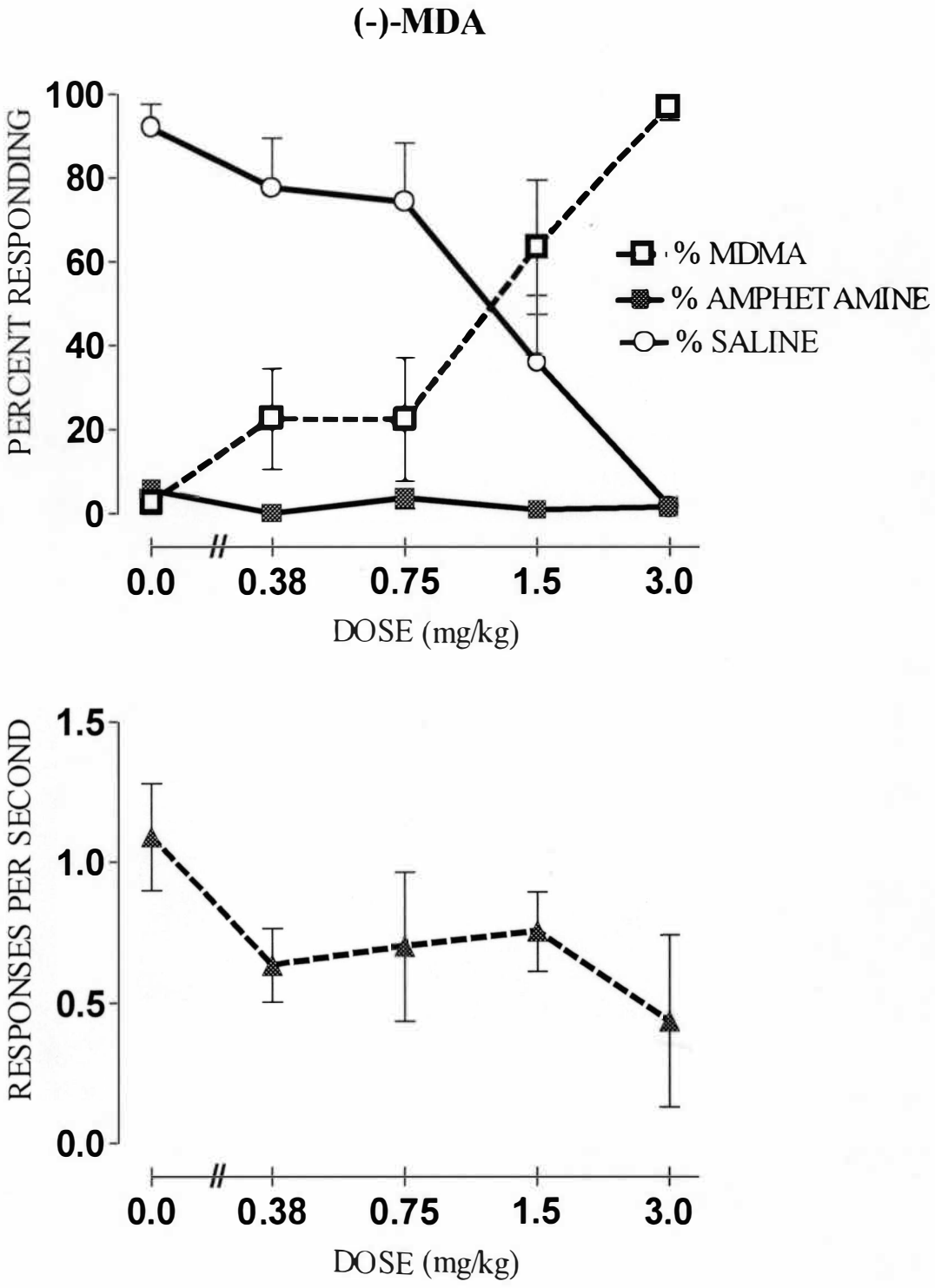

Figure 9. Results of (-)-MDA Dose-Response Tests.

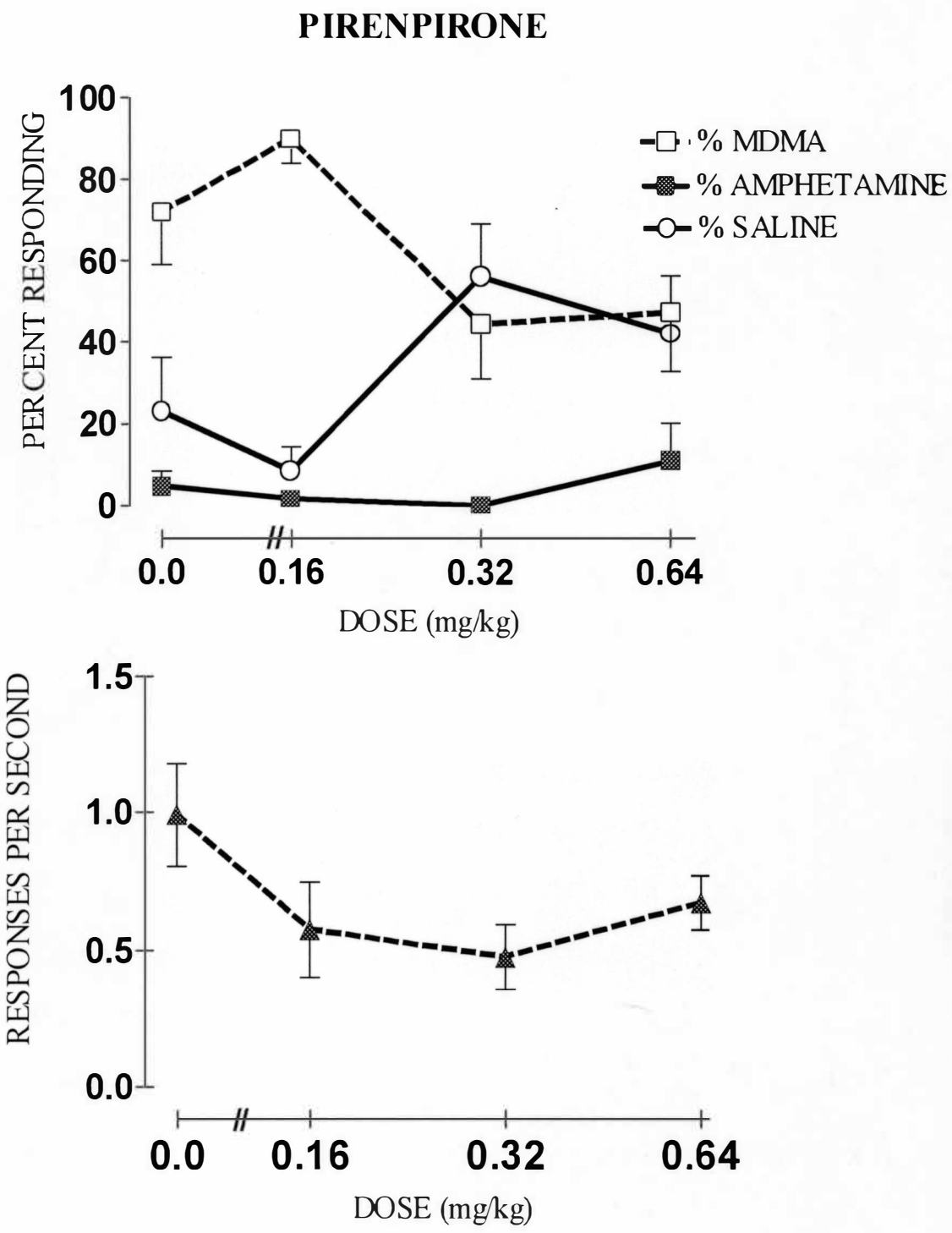

Figure 10. Results of Pirenperone Dose-Response Tests.

DISCUSSION

The present results indicate that MDMA produces discriminative stimulus effects that are distinctly different from those produced by the psychostimulant d-amphetamine. In fact, MDMA appears to produce discriminative stimulus effects that are more similar to those of the hallucinogen LSD. Moreover, these results are consistent with reports from humans that the ingestion of MDMA ("ecstasy") results in effects that are dissimilar to those of psychostimulant drugs (Grinspoon & Bakalar, 1996; Solowij, Hall, & Lee, 1992).

Although Goeters et al. (1992) reported that differential outcomes shorten the time needed for acquisition of a response, the results of the present study did not support these findings. Possible flaws in the experimental design may account for this finding. Although subjects were water deprived, outcomes consisted of diluted plain and chocolate sweetened condensed milk with the administration of drug, and water with the administration of saline. Moreover, perhaps the outcomes were not distinct enough to produce an effect. Interestingly, a difference in the MDMA stimulus generalization gradient was noted between the differential outcomes group and the control group. The data for the dose-response curves was generated after differential outcome training had been discontinued and subjects had again met criterion for discrimination. Thus, it would appear that the history of differential

training may have effected the data. A similar, but not significant, trend was observed in the d-amphetamine dose-response data. Additionally, a difference between the two groups in response-rate was significant. Overall, the utility of differential outcomes in the drug discrimination procedure is still not well established. Indeed, if the use of differential outcomes produces a more rapid rate of acquisition and an increase in the terminal accuracy of the response, it would prove to be useful in the drug discrimination assay, particularly in more complex discriminations (e.g. three-lever discriminations). The possible effects of differential outcome training on stimulus control of psychoactive compounds should also be further investigated.

It is well documented that rats exhibit stimulus generalization of cocaine to d-amphetamine, and conversely, the generalization of d-amphetamine for cocaine (Brauer, Goudie, and de Wit, 1997). The results of the present study support these findings. Numerous studies have illustrated that cocaine and d-amphetamine produce stimulus effects primarily through their actions on dopamine (DA) (Goudie, 1991; Ho and Huang, 1975; Nielsen and Jepsen, 1985; Wise, 1984; Woolverton, 1984; Young and Wise, 1975, 1976). The results of the present study support the role of DA in the stimulus effects of d-amphetamine. Although MDMA is also a potent DA releaser, the present data indicate that its discriminative stimulus effects are clearly dissociable from those of d-amphetamine. This suggests that MDMA's discriminative stimulus effects are not solely mediated by DA, but may also involve other mechanisms.

The fact that LSD produced nearly complete substitution for MDMA in the present study suggests that the discriminative stimulus effects of MDMA involve

serotonergic mechanisms. The role of serotonin in LSD discrimination is well documented (Appel, Baker, Barrett, Broadbent, Michael, Riddle, and Groll, 1991; Cunningham and Appel, 1987). Although group statistics did not illustrate complete generalization to (±)-MDMA when LSD was administered, eleven of the sixteen subjects responded at 90% or greater on the MDMA appropriate lever at the 0.08 mg/kg dose. Oberlander and Nichols (1988) reported that in rats trained to discriminate (±)-MDMA from saline, LSD produced only partial generalization. Baker, Broadbent, Michael, Matthews, Metosh, Saunders, West and Appel (1995) reported that LSD produced generalization to the negative isomer of MDMA but not the positive isomer. The results of the present study support the results of Oberlander and Nichols (1988) and demonstrate the stimulus effects of LSD to be approximate to those of MDMA.

Fenfluramine, a relatively potent serotonin releaser, produced generalization at a dose of 4.0 mg/kg. However, only two subjects out of nine tested completed the required ten responses. At the dose of 2.0 mg/kg, nearly complete substitution (75%) was observed. Again, this lends support to the role of serotonin in the discriminative stimulus effects of MDMA.

Further investigation of the role of serotonin in MDMA's discriminative stimulus effects in the present study demonstrated only partial antagonism of MDMA with the 5-HT_2 antagonist pirenpirone. This finding is consistent with previous reports that MDMA discrimination is only partially attenuated by 5-HT_2 antagonists (Schechter, 1989; Glennon et al. 1992; Baker et al. 1995). Thus, it may be concluded

that the stimulus effects of MDMA are not solely mediated by serotonin. However, Glennon et al. (1992) found more complete antagonism with a 5-HT_3 antagonists (i.e., zacopride and LY278584). Future investigations utilizing a three-choice discrimination between MDMA and amphetamine could further evaluate 5-HT_3 antagonists as well as other 5-HT antagonists.

The administration of both isomers of MDA, a structural analog of MDMA, produced dose-dependent increases in MDMA appropriate responding. (+)-MDA produced complete stimulus generalization at a dose of 1.5 mg/kg and (-)-MDA substituted at a dose of 3.0 mg/kg. These findings are consistent with a previous report by Baker et. al. (1995) that (+)-MDA exhibits stimulus generalization at lower doses than (-)-MDA in rats trained to discriminate either isomer of MDMA. These findings also support those of Glennon and Young (1984) who reported that MDMA produced stimulus generalization in rats trained to discriminate (±)-MDA. However, Glennon and Young reported that (+)-MDA produced stimulus generalization to amphetamine whereas (-)-MDA did not. Callahan and Appel (1988) reported that (-)-MDA substituted for LSD while the administration of (+)-MDA produced saline-appropriate responding in rats trained to discriminate LSD from saline. Broadbent et al. (1992) also reported that d-amphetamine produced greater drug-appropriate responding in rats trained to discriminate (+)-MDA from saline than in rats trained to discriminate (-)-MDA from saline. Additionally, Broadbent et al. observed more complete substitution with LSD for (-)-MDA than for (+)-MDA. Considered together, the results of these four studies (Glennon and Young, 1984; Callahan and

Appel 1988; Broadbent et al., 1992) suggest that the discriminative stimulus effects of (+)-MDA approximate those of amphetamine whereas the discriminative stimulus effects of (-)-MDA are more similar to those of LSD. However, the procedure used in the present study may have provided a more sensitive measure of the stimulus effects of MDA. Neither isomer of MDA produced stimulus effects similar to d-amphetamine in subjects trained to discriminate both MDMA and d-amphetamine.

Evans, Zacny, and Johanson (1990) trained pigeons to discriminate d-amphetamine, fenfluramine, and saline in a three-choice assay. Administration of (+)-MDA, (-)-MDA, and (±)-MDMA produced responding on both the d-amphetamine-appropriate and fenfluarmine-appropriate keys. (+)-MDA produced stimulus generalization to fenfluramine in two of the three pigeons tested. Administration of (-)-MDA did not produce more than 70% on either key in any of the three subjects. (±)-MDMA produced stimulus generalization to amphetamine in two of the subjects.

In summary, the utilization of a three-choice drug discrimination procedure in the present study made it possible to dissociate the discriminative stimulus effects of d-amphetamine and MDMA in rats. Because d-amphetamine is known to exert its stimulus effects primarily through dopaminergic actions, one conclusion may be that the stimulus effects of MDMA are not solely mediated through DA. In this paradigm, the discriminative stimulus control of MDMA appeared to be maintained primarily by serotonergic mechanisms. Future investigations should attempt to determine whether the discriminative stimulus effects of LSD and MDMA can be dissociated using

similar procedures. The goal of such investigations would be to assess whether dopaminergic mechanisms would be more salient in mediating MDMA's discriminative stimulus effects. If so, one might predict that d-amphetamine would exhibit stimulus generalization to MDMA in such a procedure. Such findings, along with the present results would lend support to the idea that MDMA should not be classified into the traditional drug classes of stimulants or hallucinogens, but rather be regarded as a distinct drug class (Nichols, 1986).

Appendix A

Protocol Clearance From the Institutional Animal Care and Use Committee (IACUC)

WESTERN MICHIGAN UNIVERSITY
INVESTIGATOR IACUC CERTIFICATE

Title of Project: A Three-Choice Drug Discrimination Procedure Using Differential Outcomes in Rats.

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 _____Approved with the provisions listed below

Provisions or Explanations:

IACUC Chairperson — Date 12/2/97

Acceptance of Provisions

Lisa E Baker — Date 12/2/97

Signature: Principal Investigator/Instructor

IACUC Chairperson Final Approval — Date 12/2/97

Approved IACUC Number 97-11-01

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