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Assessment of the MDA and MDMA Optical Isomers in a Stimulant-Hallucinogen Discrimination

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BAKER, L. E. AND M. M. TAYLOR. *Assessment of the MDA and MDMA optical isomers in a stimulant-hallucinogen discrimination.* PHARMACOL BIOCHEM BEHAV **57**(4) 737–748, 1997.—The phenylisopropylamine derivatives 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) have been compared to both psychostimulants and hallucinogens in drug discrimination investigations. The stereoisomers of these compounds, in particular those of MDA, appear to produce differential effects. Previous studies have demonstrated that animals trained to discriminate amphetamine from vehicle generalize to the S(+)-isomers but not the R(-)-isomers of MDA and MDMA while animals trained to discriminate LSD from saline generalize to R(-)-MDA and neither isomer of MDMA. However, animals trained to discriminate mescaline from vehicle generalize to both stereoisomers of these phenylisopropylamine derivatives. The present study consisted of two experiments in which a three-choice drug discrimination procedure was employed to compare the stereoisomers of MDA and MDMA to both amphetamine and either mescaline (experiment one) or LSD (experiment two). Sixteen male Sprague–Dawley rats were trained to discriminate S(+)-amphetamine (1.0 mg/kg) and mescaline (12.5 mg/kg) and eight rats were trained to discriminate S(+)-amphetamine (1.0 mg/kg) and LSD (0.08 mg/kg) from saline in three-choice, food reinforced drug discrimination procedures. Substitution tests were administered with the isomers of MDA and MDMA. In the second experiment, substitution tests were also administered with lower doses of each training compound and with the stimulant cocaine and the hallucinogen 2,5-dimethoxy-4-methylphenylisopropylamine (DOM). In both experiments, all of the isomers produced very few responses on the S(+)-amphetamine lever. In the first experiment, R(-)-MDA and R(-)-MDMA produced nearly complete substitution for mescaline. The results of the second experiment revealed partial substitution for LSD with both isomers of MDMA and S(+)-MDA, and nearly complete substitution with R(-)MDA for LSD. The present findings do not support previous reports that S(+)-MDMA and S(+)-MDA substitute for S(+)-amphetamine. The three-lever drug discrimination procedure may provide a more sensitive behavioral assay in which to examine the discriminative stimulus effects of drugs with compound stimulus properties. © 1997 Elsevier Science Inc.

Drug discrimination MDMA MDA Stereoisomers Amphetamine Mescaline Rats

3,4-METHYLENEDIOXYAMPHETAMINE (MDA) and 3,4-methylenedioxymethamphetamine (MDMA) are ring-substituted phenylisopropylamine derivatives with well established abuse potential. Despite their structural similarities to the psychomotor stimulant amphetamine and the hallucinogen mescaline, these compounds appear to produce unique psychoactive effects. These "designer drugs" have been reported to intensify mood, increase self-esteem, and enhance communication and intimacy, with little sensory distortion commonly associated with hallucinogens (4,17). Investigations in non-humans indicate that the discriminative stimulus properties of MDMA and MDA are similar to both stimulants and hallucinogens but comprise a more complex profile of effects than either of these traditional drug classes (1,2,7,8,9,16,19,20).

Neurochemical investigations have demonstrated that MDMA and MDA induce the presynaptic release of dopamine (DA) and serotonin (5-HT) (12,14,21–24). Analyses of the enantiomers of these compounds revealed that the S(+)-isomers are more potent DA releasers than the R(-)-isomers (10, 12, 14) and the R(-)-isomers bind to 5-HT_2 receptors with higher affinity than the S(+)-isomers (13). Behavioral investigations also indicate that S(+)-MDMA is more potent than R(-)-MDMA in disrupting operant responding in mice (18) and causing stereotyped behavior in rats (11). Considered together, the neurochemical and behavioral evidence indicate that the S(+)-isomers of MDA and MDMA are more similar to amphetamine, a potent DA releaser that also produces stereotyped behavior in rats. In fact, the stereoisomers of

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MDA and MDMA have been shown to differ in the extent to which they produce stimulus generalization in animals trained to discriminate either amphetamine or a hallucinogen. For example, S(+)-MDA substitutes for S(+)-amphetamine (7) and R(-)-MDA substitutes for the hallucinogens 2,5-dimethoxy-4-methylphenylisopropylamine (DOM) (8) and LSD (3). Although neither isomer of MDMA substitutes for DOM (8) or LSD (15), Glennon et al. (9) reported that S(+)-MDMA but not R(-)-MDMA substitutes for S(+)-amphetamine. However, Oberlender and Nichols (16) found neither isomer of MDMA to substitute for S(+)-amphetamine. Other investigators have reported that both enantiomers of MDA and MDMA substitute for mescaline, but only R(-)-MDA substitutes for LSD (3). Yet, in animals trained to discriminate the individual enantiomers of MDA (2) or MDMA (1), mescaline does not produce stimulus generalization while LSD substitutes for R(-)-MDMA and both isomers of MDA, and DOM substitutes for R(-)-MDA. Also, S(+)-amphetamine does not substitute for either MDMA enantiomer and produces only partial stimulus generalization in animals trained to discriminate S(+)-MDA (1,2).

The inconsistencies among the results described above indicate that the extent to which the discriminative stimulus properties of the MDMA and MDA isomers are amphetamine or hallucinogen-like depends on the training drug and the discrimination training procedures employed. A recent report by Young and Glennon (25) suggested that animals could be trained to discriminate the two isomers of MDA in a three-choice discrimination procedure and that S(+)-amphetamine produced S(+)-MDA-lever responses while DOM produced predominantly R(-)-MDA-lever responses. Those findings are consistent with previous reports from two lever amphetamine-vehicle and DOM-vehicle discrimination experiments (7,8). Additional investigations employing the three-choice drug discrimination procedure may further delineate the discriminative stimulus properties of the stereoisomers of MDA and MDMA. The present study employed a three-choice drug discrimination procedure in two separate experiments. In the first experiment, we attempted to train animals to discriminate the stimulus properties of both S(+)-amphetamine and mescaline, and test the individual isomers of MDA and MDMA for substitution. In the second experiment, animals were trained to discriminate both S(+)-amphetamine and LSD from saline. In addition to testing the isomers of MDA and MDMA for substitution, these animals were also tested with lower doses of each training compound as well as cocaine and DOM.

EXPERIMENT ONE

Methods

Subjects. Sixteen male Sprague–Dawley rats (Harlan Breeding Laboratories, Indianapolis, IN) aged 6 to 8 mo and weighing 350–400 g at the beginning of the study served as subjects. The subjects had been previously exposed to operant training on a single lever in an undergraduate psychology lab. All subjects were drug naive prior to the onset of the present study. The animals were individually housed in wire mesh cages, in a colony maintained on a 12-h light (0700 to 1900)/12-h dark cycle and at constant temperatures (20–22°C). Water was provided ad libitum and commercial rat chow was rationed to maintain animals at approximately 85% of their free feeding weights throughout the study.

Apparatus. Training and testing were conducted in eight standard operant chambers (MED Associates Inc., St. Albans, VT, ENV-001), housed in sound- and light-attenuating shells, which provided ventilation and masking noise. Each chamber contained an overhead 28 v house light and a dipper (0.1 ml) mounted equidistant between the left and right levers and below the center lever. A Zenith 320-SX computer was programmed using MED-PC instrumentation and software (MED Associates Inc., St. Albans, VT, version 2.0) to control experimental events and data collection.

Drugs. Mescaline and S(+)-amphetamine were obtained from Sigma Chemical Company (St. Louis, MO) and from the National Institute on Drug Abuse (Rockville, MD). The MDMA and MDA isomers were obtained from the National Institute on Drug Abuse (Rockville, MD). All doses were expressed as the salt. All drugs were dissolved in 0.85% physiological saline and administered intraperitoneally.

Procedures.

Discrimination training: Subjects were trained to discriminate S(+)-amphetamine (1.0 mg/kg) and mescaline (10 mg/kg) from saline in a three-choice drug discrimination procedure under a fixed ratio 20 (FR 20) schedule of reinforcement. Following saline injections, all subjects were reinforced for responses on the center lever with diluted sweetened condensed milk (1 part milk: 2 parts water). Half the subjects were reinforced for responses on the left lever following S(+)-amphetamine injections and for responses on the right lever following mescaline injections. Conditions were reversed for the remaining animals. Drug or saline injections were administered intraperitoneally (IP), 15 min. prior to 20 min. training sessions. Training sessions were conducted at the same time of day six days per week (Mon.–Sat.).

For each condition, training began under a fixed ratio 1 (FR 1) schedule. When responding was consistent and stable, the FR was gradually increased from 1 to 20. Reinforcement was contingent upon 20 consecutive responses on the correct lever. Incorrect responses on either lever reset the response counter and no reinforcement was delivered until 20 consecutive responses were made on the correct lever. To reduce the effects of olfactory stimuli on response choice, all levers were wiped with isopropyl alcohol between sessions (6). During the first week of training, S(+)-amphetamine and saline training sessions were alternated. The following week, mescaline and saline training sessions were alternated. Subsequently, a semi-random schedule of training conditions was presented, such that an equivalent number of mescaline and S(+)-amphetamine training sessions occurred over each two week period and no animal received more than three consecutive drug training sessions.

Because most animals were not reliably discriminating mescaline from saline after 80 training sessions but were reliably discriminating S(+)-amphetamine, the frequency of S(+)-amphetamine training sessions was reduced to at least once within each three week period, while mescaline and saline training conditions were alternated on a semi-random basis. After 166 total training sessions, the mescaline dose was increased to 12.5 mg/kg. After 173 sessions, the frequency of S(+)-amphetamine training sessions was increased to equal the frequency of mescaline training sessions.

Stimulus substitution testing: Percent correct lever choice prior to the first reinforcer of each training session was used to determine discrimination acquisition. Animals that achieved a mean of at least 80% correct lever choice over a period of 10 consecutive training sessions were administered substitution tests with S(+)-MDA (0.312–1.25 mg/kg) and R(-)-MDA (0.312–1.25 mg/kg) or S(+)-MDMA (0.312–1.25 mg/kg) and R(-)-MDMA (0.875–3.5 mg/kg). Test doses were chosen based

on previous studies in which animals were trained to discriminate each of these isomers (1,2). Animals were randomly assigned to receive substitution tests with either the MDA or MDMA isomers. The order of dose presentation was counterbalanced among the animals administered the MDA isomers ($n = 6$) and those administered the MDMA isomers ($n = 6$). Substitution tests were conducted under extinction and ended when 20 consecutive responses were completed on any lever or after 20 min, which ever occurred first. Substitution tests were administered every third or fourth day in animals that maintained the minimum criterion of 80% correct during training sessions.

Data Analysis. For each dose tested, the mean percent of total responses on each lever was calculated and plotted for visual analysis. Since the greatest percent of total responses occurred on the mescaline lever, two factor repeated measures analyses of variance were conducted on the percent of total responses made on the mescaline lever during substitution tests with the MDA isomers and during substitution tests with the MDMA isomers. The two factors were isomer (S(+) vs. R(-)) and dose (3 dose levels and vehicle control). Vehicle control values were calculated for each animal by averaging the percent of total responses on the mescaline lever prior to the delivery of the first reinforcer during the saline training sessions that occurred immediately before each substitution test. Complete stimulus generalization (substitution) was defined as a mean of at least 80% of the total responses on any particular lever. Partial generalization was said to occur if any particular dose produced less than 80% responding on either drug lever, but this percent was significantly different from saline control. Response rate (responses per second) during substitution tests were also calculated, plotted and subjected to two factor (isomer, dose) repeated measures ANOVAs. Vehicle control values for response rate were calculated for each animal by averaging the response rate during saline training sessions that occurred immediately before each substitution test.

RESULTS

S(+)-amphetamine acquired discriminative control over responding in all but one animal within an average of 52 (SEM = 1.98) total training sessions (range, 44-76). During mescaline training sessions, incorrect responses were made exclusively on the saline-appropriate lever. Acquisition of the mescaline-saline discrimination required additional training and an increase in the training dose to 12.5 mg/kg. The discrimination criterion (individual means of at least 80% correct prior to first reinforcer over 10 consecutive training sessions) was met in 12 of the 16 animals within an average of 209 (SEM = 1.63) total training sessions (range, 201-222).

Six animals completed substitution tests with S(+)-MDA and the two lower doses of R(-)-MDA, five of these animals completed the highest dose of R(-)-MDA. Another group of six animals were administered substitution tests with both isomers of MDMA, although each data point in Fig. 1 represents a mean from five animals. Four animals completed all test doses of both MDMA isomers, one animal completed tests with all doses of S(+)-MDMA and one dose of R(-) MDMA and one animal completed tests with two doses of R(-)MDMA.

Substitution tests with each isomer of MDA produced very little S(+)-amphetamine-lever responding (See Fig. 1). Approximately 50% of the responses occurred on the mescaline lever with the 0.312 and 1.25 mg/kg doses of S(+)-MDA and about 60% of the responses occurred on the saline lever with the 0.625 mg/kg dose. S(+)-MDA substituted completely for mescaline in four of the six animals, two at the lowest dose, one at the middle and highest dose, and one at the highest dose. No dose of S(+)-MDA substituted completely for S(+)-amphetamine; at 1.25 mg/kg one animal allocated 53% of its responses on the S(+)-amphetamine lever, but the rest of the animals made less than 15% of their responses on the S(+)-amphetamine lever. At the highest dose tested (1.25 mg/kg), R(-)-MDA produced a mean of 79% mescaline-lever responses (see Fig. 1, right). Two of the six animals tested at this dose generalized completely (90%, 100%), while two animals emitted 75% of their responses on the mescaline lever. In three of the animals, mescaline-lever responding generalized completely to a dose of 0.625 mg/kg R(-)MDA. In contrast, all animals allocated less than 15% of their responses on the S(+)-amphetamine-lever with all doses of R(-)-MDA.

Visual inspection of the dose effect curves revealed that neither isomer of MDA substituted for S(+)-amphetamine and R(-)-MDA substituted partially for mescaline. A two-factor repeated measures ANOVA revealed a significant effect of dose [$F(3, 19) = 4.76, p < 0.05$] but no significant effect of isomer [$F(1, 19) = 1.18, p > 0.10$] on percent mescaline-lever responses. Rate was also substantially decreased by the MDA isomers compared to vehicle control levels. A two factor repeated measures ANOVA on response rate revealed a significant dose effect [$F(3, 18) = 16.51, p < 0.001$] but no significant effect of isomer [$F(1, 18) = 1.56, p > 0.10$].

The results of substitution tests with each isomer of MDMA are illustrated in Fig. 2. At all doses of each isomer tested, the mean percent of S(+)-amphetamine lever responses was less than 20%. Only two individual animals allocated more than 20% of their responses on the S(+)-amphetamine lever; one animal made 46% and one animal made 27% of its responses on the S(+)-amphetamine lever with a dose of 1.25 mg/kg S(+)-MDMA. S(+)-MDMA produced about equal responding on saline and mescaline levers. However, R(-)-MDMA produced a greater percent of total responses on the mescaline lever than on the saline lever. The 3.5 mg/kg dose produced a mean of 74% of the responses on the mescaline lever. At all doses, R(-)-MDMA substituted completely for mescaline in at least two of the five animals tested, although not in the same two animals at all three doses.

Visual inspection of the dose effect curves revealed that neither isomer of MDMA substituted for S(+)-amphetamine and R(-)-MDMA produced partial substitution for mescaline. A two factor repeated measures ANOVA on percent mescaline-lever responses revealed a significant dose effect [$F(3,14) = 4.06, p < 0.05$] and a significant effect of isomer [$F(1, 14) = 8.99, p < 0.05$], but no significant interaction between dose and isomer. Both MDMA isomers decreased the overall rate of responding compared to vehicle control. A two factor repeated measures ANOVA on response rate revealed a significant dose effect [$F(3, 15) = 23.47, p < 0.001$] but no significant effect of isomer [$F(1, 15) = 1.90, p > 0.10$].

DISCUSSION

This study is the first to document that animals can be trained to discriminate both S(+)-amphetamine and mescaline in a three-choice drug discrimination procedure. However, the number of training sessions required to attain the discrimination criterion was approximately seven times longer than that reported in two-choice discrimination experiments with either mescaline or S(+)-amphetamine (3, 16). S(+)-amphet-

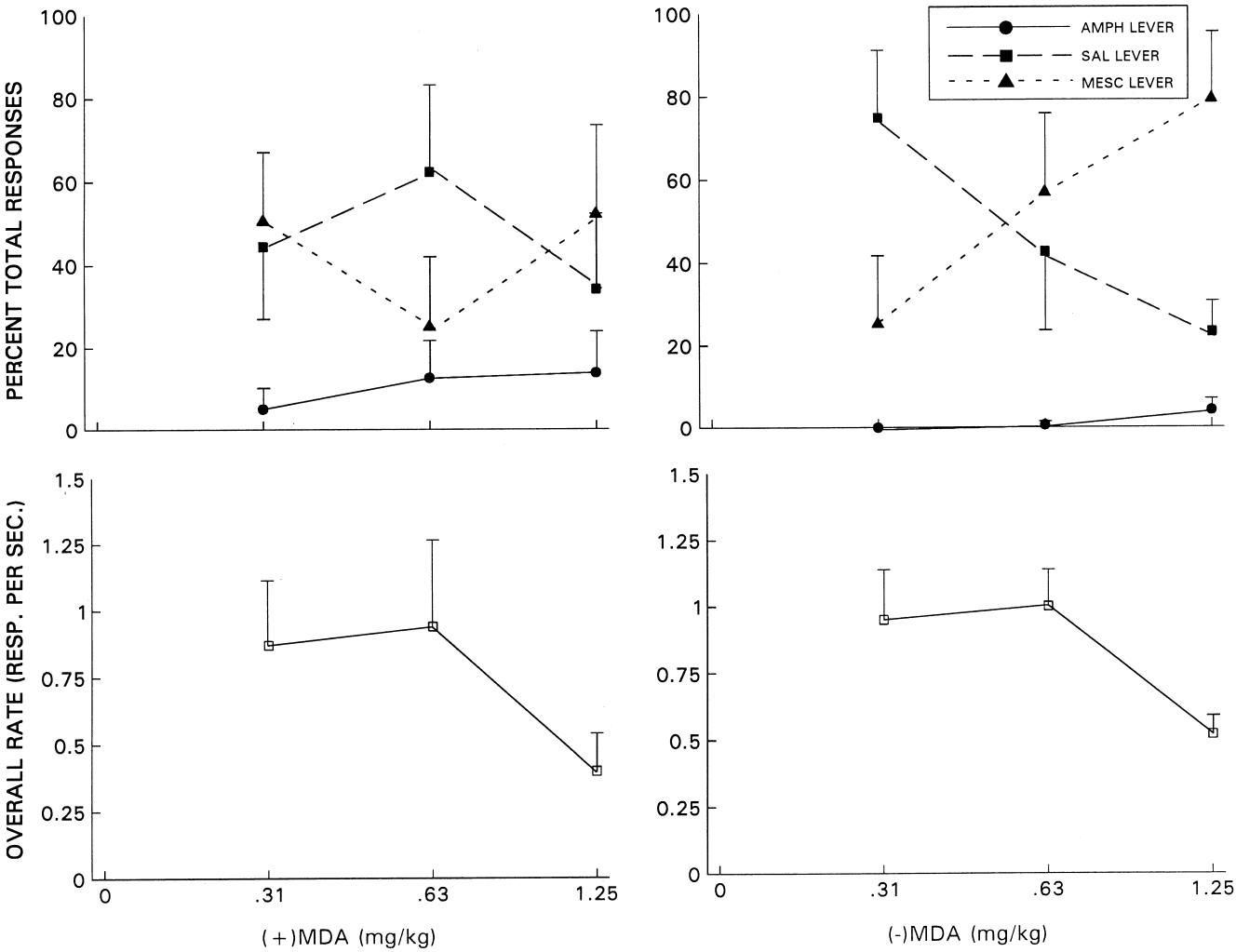

FIG. 1. Dose response functions for S(+)-MDA (left) and R(-)-MDA (right). Percent total responses on the S(+)-amphetamine, saline and mescaline levers (top) and overall rate on all three levers (bottom) are indicated. (n = 6 at all doses of S(+)-MDA and 0.312, 0.625 mg/kg R(-)-MDA; n = 5 at 1.25 mg/kg R(-)-MDA.)

amine clearly gained discriminative control over responding more rapidly than mescaline. During amphetamine training sessions most animals responded exclusively on the amphetamine lever. During mescaline training sessions, incorrect responses were always on the saline lever and during saline training sessions, errors occurred most frequently on the mescaline lever. It was necessary to increase the training dose of mescaline to establish its discriminative stimulus control. Still, only 12 of the 16 animals met criterion under the conditions employed in this study.

The results of stimulus substitution tests in these animals suggest that both stereoisomers of MDA and MDMA are dissimilar to S(+)-amphetamine. These findings are somewhat surprising in light of previous reports that S(+)-MDA and S(+)-MDMA substitute for S(+)-amphetamine (7, 9) in rats. However, at least one other report (16) exists that S(+)-MDMA does not substitute for S(+)-amphetamine. Furthermore, animals trained to discriminate the S(+) isomer of MDMA (1) or MDA (2) do not generalize to S(+)-amphetamine, although S(+)-amphetamine did produce a significant amount of S(+)-MDA-appropriate responding (partial substitution) in the study by Broadbent et al. (2).

The present results also suggest that the discriminative stimulus effects of the R(-)-isomers of MDA and MDMA are similar to those of mescaline. Although there was not a statistically significant difference between the two MDA isomers in generalization tests, the R(-)-isomers did tend to produce a greater percent of mescaline lever responses than the S(+)-isomers. This result is consistent with neuropharmacological evidence that the R(-)isomers have a higher affinity for 5-HT_2 receptors than the S(+)-isomers (13). While complete stimulus generalization to either component of the three-choice discrimination was not achieved with the R(-)-isomers in the present study, higher doses might substitute for mescaline. Previous reports of two-choice discrimination experiments indicate that both optical isomers of MDA and MDMA substitute for mescaline (10 mg/kg) (3). Moreover, stimulus generalization occurred with doses of the isomers that did not substitute for mescaline in the present study. However, the training dose of mescaline was increased to 12.5 mg/kg in the

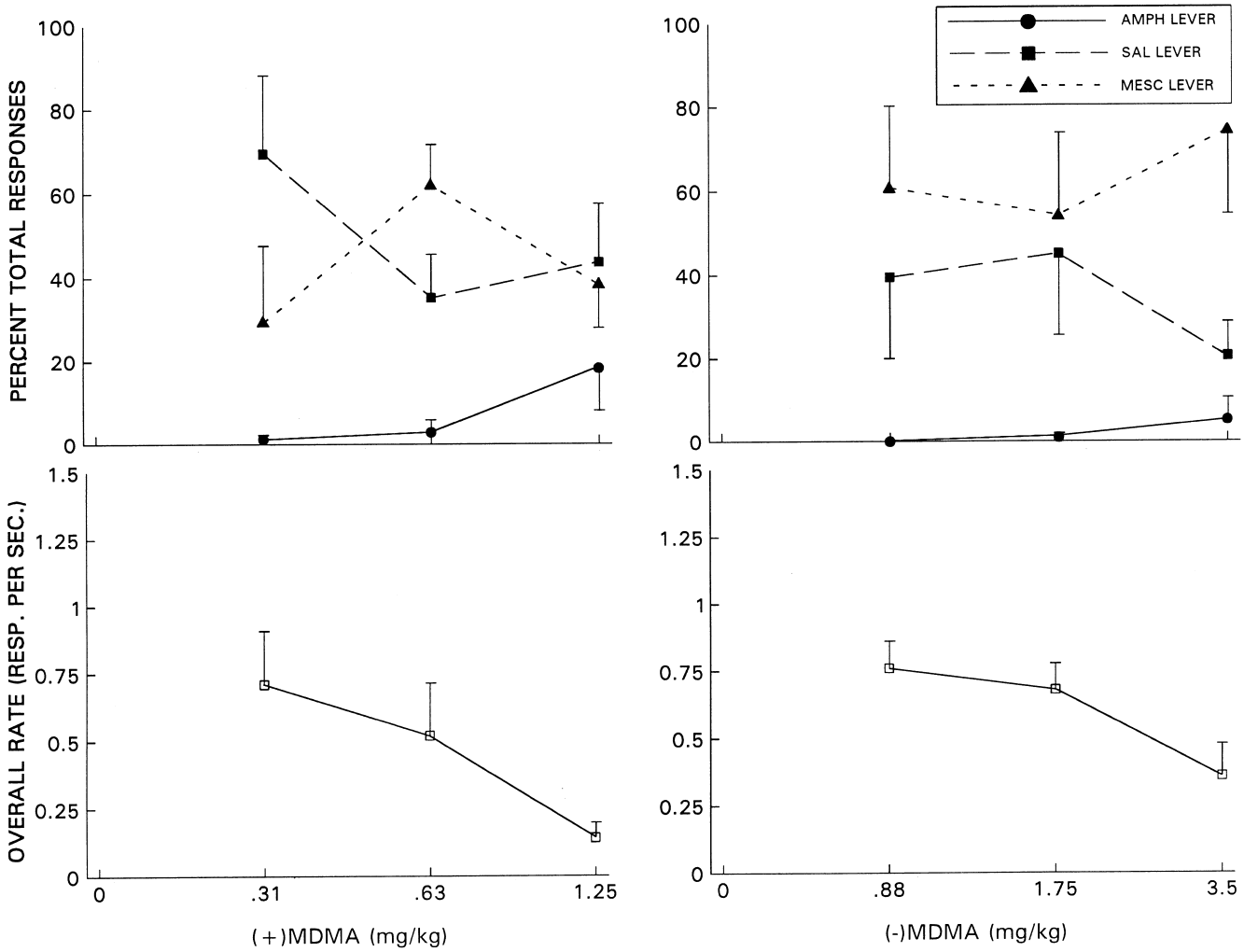

FIG. 2. Dose response functions for S(+)-MDMA (left) and R(-)-MDMA (right). Percent total responses on the S(+)-amphetamine, saline and mescaline levers (top) and overall rate on all three levers (bottom) are indicated. (n = 5 at all doses of both isomers.)

present study, which could account for the difference in the amount of stimulus generalization observed in the study reported by Callahan and Appel (3) and that observed in the present one. When the individual MDA or MDMA isomers are employed as training drugs, mescaline does not substitute for any of these substances while LSD substitutes for R(-)-MDMA and both MDA isomers (1, 2). Thus, the degree of similarity between these isomers and various hallucinogens appears to depend on which drug (and dose) is employed as the discriminative stimulus during training.

The extent to which novel compounds substitute for drugs that have acquired discriminative control clearly depends on the number of drugs employed as discriminative stimuli. When rats are trained to discriminate S(+)-amphetamine only (e.g., 7, 9), S(+)-MDA and perhaps S(+)-MDMA substitute for S(+)-amphetamine but the R(-) isomers do not. When rats are trained to discriminate mescaline only (3), they generalize to both S(+) and R(-) isomers of MDA and MDMA. However, when animals are trained to discriminate the effects of mescaline and S(+)-amphetamine, very little S(+)-amphetamine-lever responding is elicited by either enantiomer. However, without dose response functions for amphetamine or mescaline, these conclusions must be considered with caution. Since the subjects were 16 mo old by the time the discrimination criterion was met, and due to other practical concerns such as laboratory re-location, we were unable to administer substitution tests with lower doses of the training compounds or higher doses of the MDA and MDMA isomers. Therefore, a second experiment was conducted to further assess the stimulus effects of these isomers in a three-choice discrimination. Because of the extensive amount of training required to reach the discrimination criterion in the first experiment (over 200 training sessions), animals were trained to discriminate a different hallucinogen, LSD from both S(+)-amphetamine and saline in the second experiment.

EXPERIMENT TWO

Method

Subjects. Eight male Sprague–Dawley rats (Harlan Breeding Laboratories, Indianapolis, IN) aged 6 to 8 mo and weighing 350–400 g at the beginning of the study served as

subjects. Animals were drug naive at the onset of the study and housed in the same manner described in experiment one.

Apparatus. The same apparatus described in experiment one was employed in experiment two.

Drugs. LSD, S(+)-amphetamine, DOM, cocaine, and the isomers of MDA and MDMA were obtained from the National Institute on Drug Abuse (Rockville, MD). All doses were expressed as the salt. All drugs were dissolved in 0.85% physiological saline and administered intraperitoneally.

Procedures.

Discrimination training. Training procedures were similar to those described in experiment one. Subjects were trained to discriminate S(+)-amphetamine (1.0 mg/kg) and LSD (0.08 mg/kg) from saline in a three-choice drug discrimination procedure under a resetting fixed ratio 20 (FR 20) schedule of reinforcement. Following saline injections, all subjects were reinforced for responses on the center lever with diluted sweetened condensed milk (1 part milk: 2 parts water). Half the subjects were reinforced for responses on the left lever following S(+)-amphetamine injections and for responses on the right lever following LSD injections. Conditions were reversed for the remaining animals. Drug or saline injections were administered intraperitoneally (IP), 15 min. prior to 20 min. training sessions. Training sessions were conducted at the same time of day six days per week (Mon.–Sat.). See experiment one for additional details regarding training procedures.

Stimulus substitution testing. For each subject, the percent correct lever choice prior to the first reinforcer of each training session was used to determine discrimination acquisition. Subjects that achieved a mean of at least 85% correct lever choice over a period of 10 consecutive training sessions were administered substitution tests with the following compounds: amphetamine (0.25–2.0 mg/kg), LSD (0.02–0.16 mg/kg), cocaine (1.25–15 mg/kg), DOM (0.5–1.5 mg/kg), S(+)-MDA (0.312-2.5 mg/kg), R(-)-MDA (0.312-2.5 mg/kg), S(+)-MDMA (0.312-2.5 mg/kg), R(-)-MDMA (0.875-5.0 mg/kg) and saline. With the exception of the highest dose of each isomer of MDMA and MDA (see below), the doses of each drug were administered in a semi-random order across subjects. Substitution tests were conducted under extinction and ended when 20 consecutive responses were completed on any lever or after 20 min, which ever occurred first. Substitution tests were administered every third or fourth day in animals that maintained the minimum criterion of 85% correct during training sessions. Because of this requirement, only five of the eight subjects were administered some of the test doses near the end of the study. For example, three animals were not administered the highest dose of each isomer of MDA and MDMA. These doses were administered in the last four test sessions in random order among subjects. Because these doses were behaviorally disruptive, subjects were given two recovery days before training resumed.

Data analysis. For each dose tested, the mean percent of total responses on each lever was calculated and plotted for visual analysis. Response rate was expressed as the number of total responses (on all three levers) per second. The mean response rate was calculated at each dose tested and also plotted for visual analysis. Data from subjects that emitted fewer than 20 total responses during a test session were excluded from the analyses. For the amphetamine, LSD, cocaine and DOM dose response functions, one factor analyses of variance were conducted to determine the statistical significance of dose on percent amphetamine-lever or percent LSD-lever responding. For the dose response functions with the optical isomers of MDA and MDMA, two factor (dose, isomer) analyses of variance were conducted on percent amphetamine-lever and percent LSD-lever responding. Complete stimulus generalization (substitution) was defined by at least 80% of the total responses on any particular lever. Partial generalization was said to occur if any particular dose produced less than 80% responding on either drug lever, but this percent was significantly different from saline control.

RESULTS

Each of the eight subjects' behavior came under stimulus control of all three training conditions and the discrimination criterion was met by all eight subjects within an average of 100 training sessions. When LSD control tests were conducted, the condition each subject received during the previous session appeared to modulate the results. For instance, in the two subjects for which LSD control tests followed LSD training sessions, the percent of total responses on the LSD-appropriate lever was much lower than in the subjects that received S(+)-amphetamine or saline during the preceding session. Therefore, control tests with the training dose of LSD were conducted following each of the three training conditions. The control values at this dose represent a mean of three tests for seven animals and a mean of two tests for one animal. The group mean was 92% following S(+)-amphetamine training sessions ($n = 8$), 76% following saline training sessions ($n = 8$) and only 53% following LSD training sessions ($n = 7$). The overall mean (81%) was calculated from the average of three tests for each subject. The other doses of LSD and the other test drugs were not tested following each of the three training conditions. However, for each dose tested, approximately one-third of the subjects were tested after each of the three training conditions. No consistent differences were observed between the results of other tests administered after each training condition.

Figure 3 illustrates the dose response curves for both training drugs. LSD produced dose dependent increases in LSD-appropriate responding (graph A). The training dose produced a mean of 81% LSD-appropriate responding. A higher dose of LSD (.16 mg/kg) was also tested for substitution and was found to produce a mean of only 60% LSD-appropriate responding. A one factor repeated measures ANOVA on percent LSD-lever responding revealed a significant dose effect [$F(4,28) = 4.50, p < 0.01$]. A one factor repeated measures ANOVA on LSD response rate revealed a nonsignificant dose effect [$F(4,28) = 0.33, p > 0.10$]. Figure 3 also depicts the dose response curve for S(+)-amphetamine (graph B). A mean of 82% was obtained when the training dose of S(+)-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 FR 20 requirement when given this dose of S(+)-amphetamine. A one factor repeated measures ANOVA on percent S(+)-amphetamine-lever responding revealed a significant effect of dose [$F(3, 21) = 11.34$, $p < 0.001$]. There was not a significant dose effect on response rate during S(+)-amphetamine tests [$F(3, 21) = 2.38$, $p = 0.09$], although the 2.0 mg/kg dose was not included in the statistical analyses.

To confirm that a hallucinogen-stimulant discrimination had been established with the present three-choice discrimination procedure, DOM ($n = 7$) and cocaine ($n = 5$) were tested for stimulus generalization. Figure 4 depicts the dose response functions for both DOM (graphs A and C) and cocaine (graphs B and D). A visual analysis revealed that DOM produced dose dependent increases in LSD-appropriate responding. An

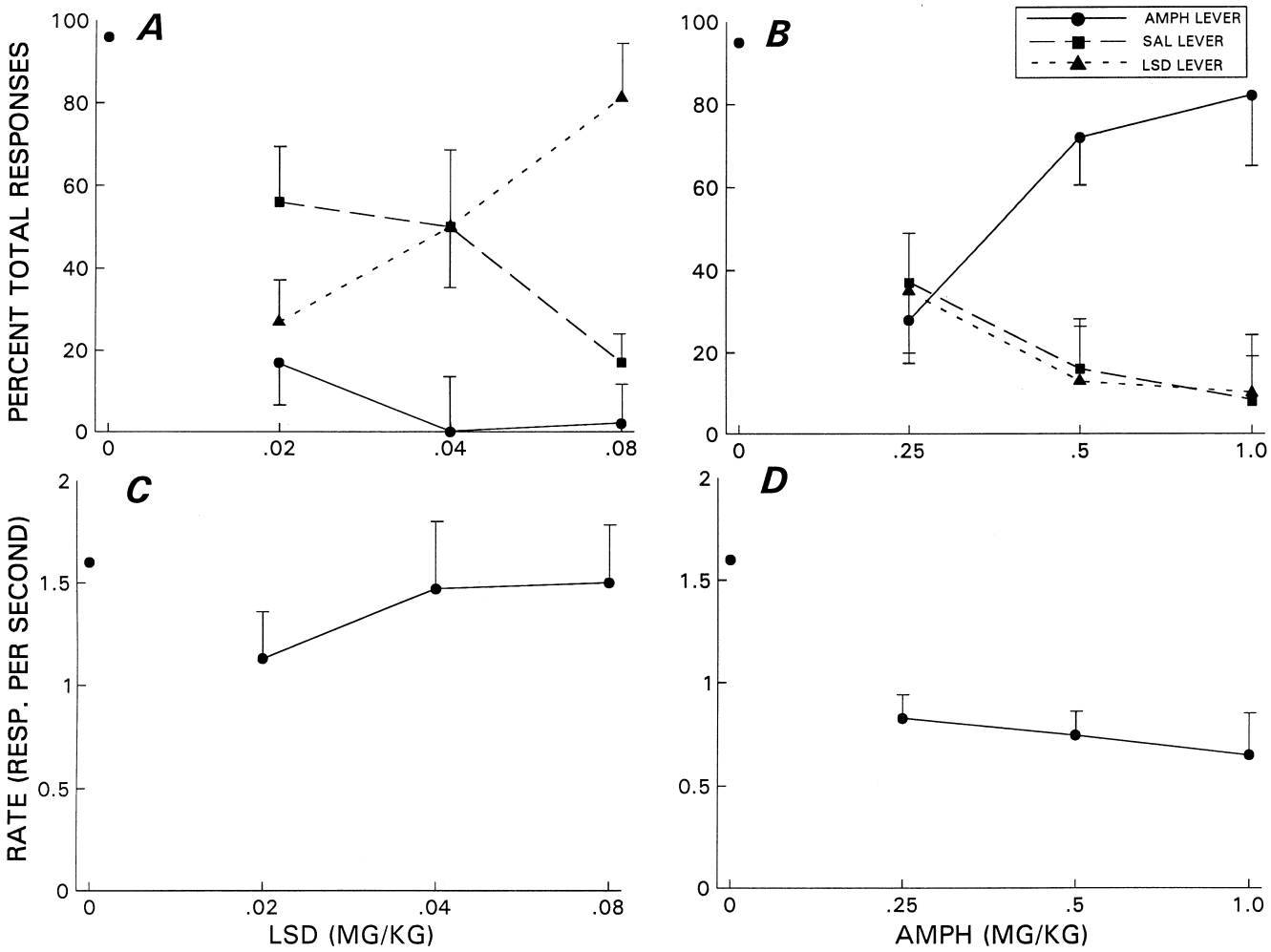

FIG. 3. Dose response functions for LSD and S(+)-amphetamine (n = 8 at all doses). Graphs A and B depict percent total responses on each lever for LSD and S(+)-amphetamine, respectively. Graphs C and D depict response rate for LSD and S(+)-amphetamine, respectively. The ● symbol along the Y axis indicates the saline control value.

overall mean of 87% was obtained at the highest dose tested. A one factor repeated measures ANOVA revealed a significant dose effect on percent LSD-lever responding [$F(3,18) = 10.11$, $p < 0.001$] but a nonsignificant effect on response rate [$F(3, 18) = 1.01$, $p > 0.10$]. A visual analysis also revealed that cocaine produced dose dependent increases in S(+)-amphetamine-appropriate responding. Since little variation in S(+)-amphetamine-appropriate responding occurred with 2.5, 5.0 and 10.0 mg/kg cocaine (81%, 80%, and 83% respectively), a lower dose (1.25 mg/kg) and a higher dose (15.0 mg/kg) of cocaine were also tested. All of the subjects emitted at least 90% of their responses on the S(+)-amphetamine lever at the 15 mg/kg dose (overall mean 96%). A one factor repeated measures ANOVA revealed a significant dose effect on percent amphetamine-appropriate responding [$F(5, 20) = 11.16$, $p < 0.001$] but not on response rate [$F(5,20) = 1.30, p > 0.10$].

Figure 5 illustrates the dose response curves for the optical isomers of MDA. Visual inspection of these dose response curves revealed that neither of these isomers substitute for S(+)-amphetamine. However, S(+)-MDA (0.63 mg/kg) did substitute for S(+)-amphetamine in two subjects. These two subjects emitted 100% of their responses on the S(+)-amphetamine-appropriate lever, although only one of these subjects exhibited stimulus generalization to S(+)-amphetamine with 1.25 mg/kg S(+)-MDA. Moreover, the 1.25 mg/kg dose of S(+)-MDA produced partial generalization to LSD (66%). Five of the eight subjects were tested with a 2.5 mg/kg dose of S(+)-MDA, but only two of these subjects made at least 20 total responses during the 20 min test session. One subject emitted 81% of its responses on the S(+)-amphetamine lever and one emitted 68% of its responses on the saline lever. Since this dose was highly disruptive, higher doses of S(+)-MDA were not tested. R(-)-MDA produced dose dependent increases in LSD-appropriate responding, and virtually no responding on the S(+)-amphetamine lever. Five of the eight subjects were administered substitution tests with 2.5 mg/kg R(-)-MDA; four of these subjects exhibited complete stimulus generalization to LSD at this dose. A two factor (dose, isomer) repeated measures ANOVA revealed a significant dose effect [$F(4, 61) = 3.77$, $p < 0.01$] but a nonsignificant effect of MDA isomer [$F(1, 61) = 0.99$, $p = 0.32$] on percent LSD-lever responses. A similar analysis on percent S(+)-amphetamine-lever

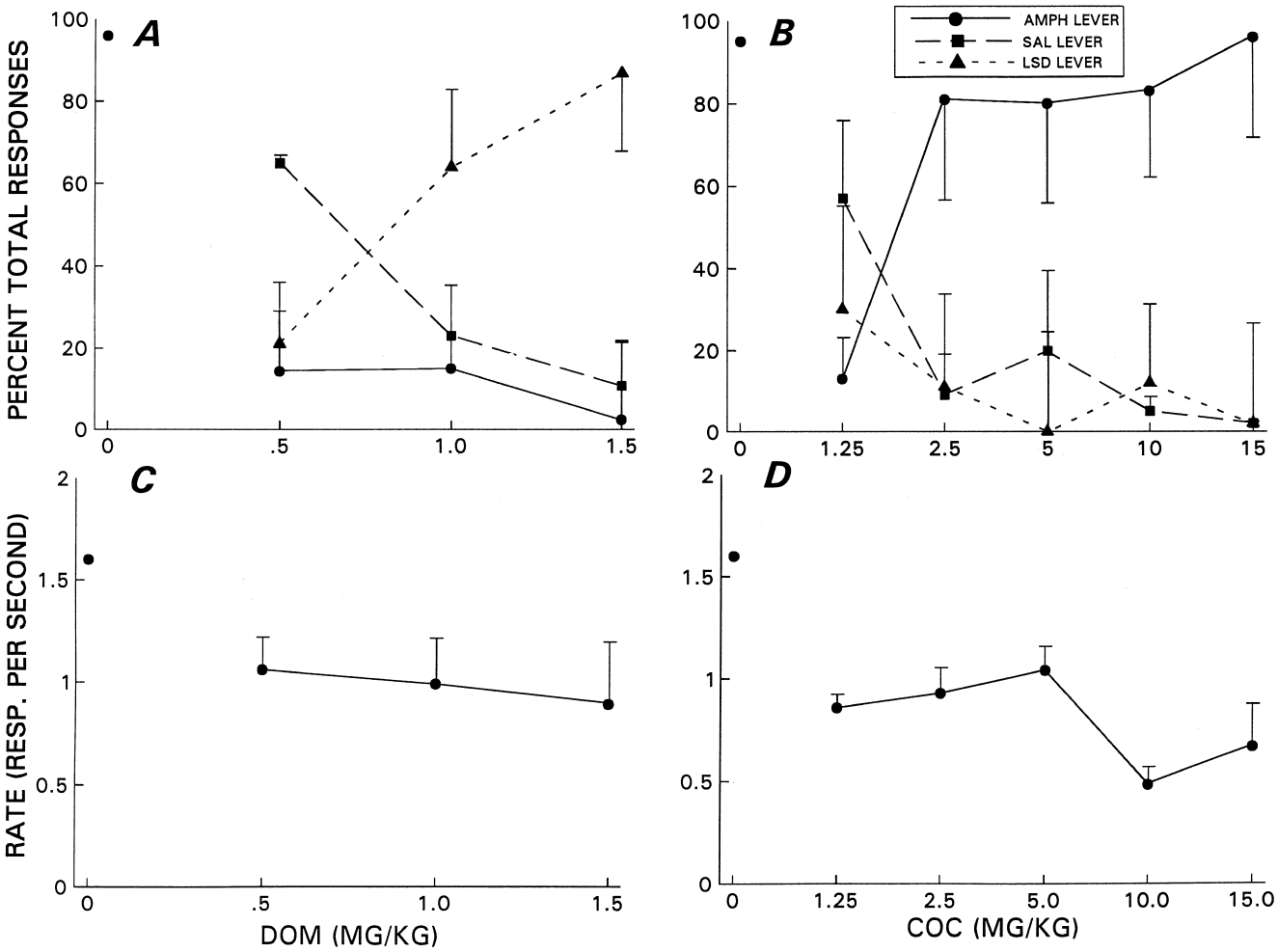

FIG. 4. Dose response functions for DOM (n = 7) and cocaine (n = 5). Graphs A and B depict percent total responses on each lever for DOM and cocaine, respectively. Graphs C and D depict response rate for DOM and cocaine, respectively. The ● symbol along the Y axis indicates the saline control value.

responses revealed a significant dose effect [$F(4, 61) = 3.39$, $p < 0.05$] and a significant effect of isomer [$F(1, 61) = 10.88$, $p < 0.01$], but a nonsignificant interaction [$F(4, 61) = 2.27$, $p = 0.07$].

Figure 6 illustrates the dose response curves for the MDMA isomers. Similar to the results obtained with the MDA isomers, very little S(+)-amphetamine-appropriate responding occurred in substitution tests with either MDMA isomer. Although 1.25 mg/kg S(+)-MDMA produced 71% LSD-appropriate responding and less than 10% S(+)-amphetamine-appropriate responding, 2.5 mg/kg produced about equal amounts of responding on each lever. However, this dose was highly disruptive. R(-)-MDMA produced less than 2% of the total responses on the S(+)-amphetamine lever at all doses tested, but produced dose dependent increases in LSD lever responses. However, only partial substitution for LSD occurred with 3.5 mg/kg and a higher dose (5.0 mg/kg) completely disrupted behavior. None of the animals tested at this dose completed 20 responses during the 20 min test session. The subjects laid flat on their stomachs and emitted very few responses. Also, a clear fluid was observed dripping from the nose and mouth for several hours following the administration of this dose. In fact, this was observed with the highest dose of all the isomers tested. A two factor repeated measures ANOVA on percent LSD-lever responses revealed a significant dose effect [$F(3, 56) = 5.03$, $p < 0.005$], although the effect of isomer was not significant [$F(1, 56) = 1.32$, $p = 0.26$]. A two factor repeated measures ANOVA also revealed nonsignificant dose [$F(3, 56) = 1.44$, $p = 0.24$] and isomer [$F(1, 56) = 0.35$, $p = 0.56$] effects on percent S(+)-amphetamine-lever responses.

DISCUSSION

This experiment provides additional evidence that rats can be trained to differentially respond to the subjective effects of a hallucinogen (in this case, LSD) and a stimulant in a three-choice drug discrimination assay. The discrimination criterion was met in approximately half the number of sessions required in the first experiment, in which mescaline was used as the hallucinogenic stimulus. Furthermore, dose response functions with the two training drugs and with cocaine and DOM support the conclusion that a hallucinogen-stimulant discrimi-

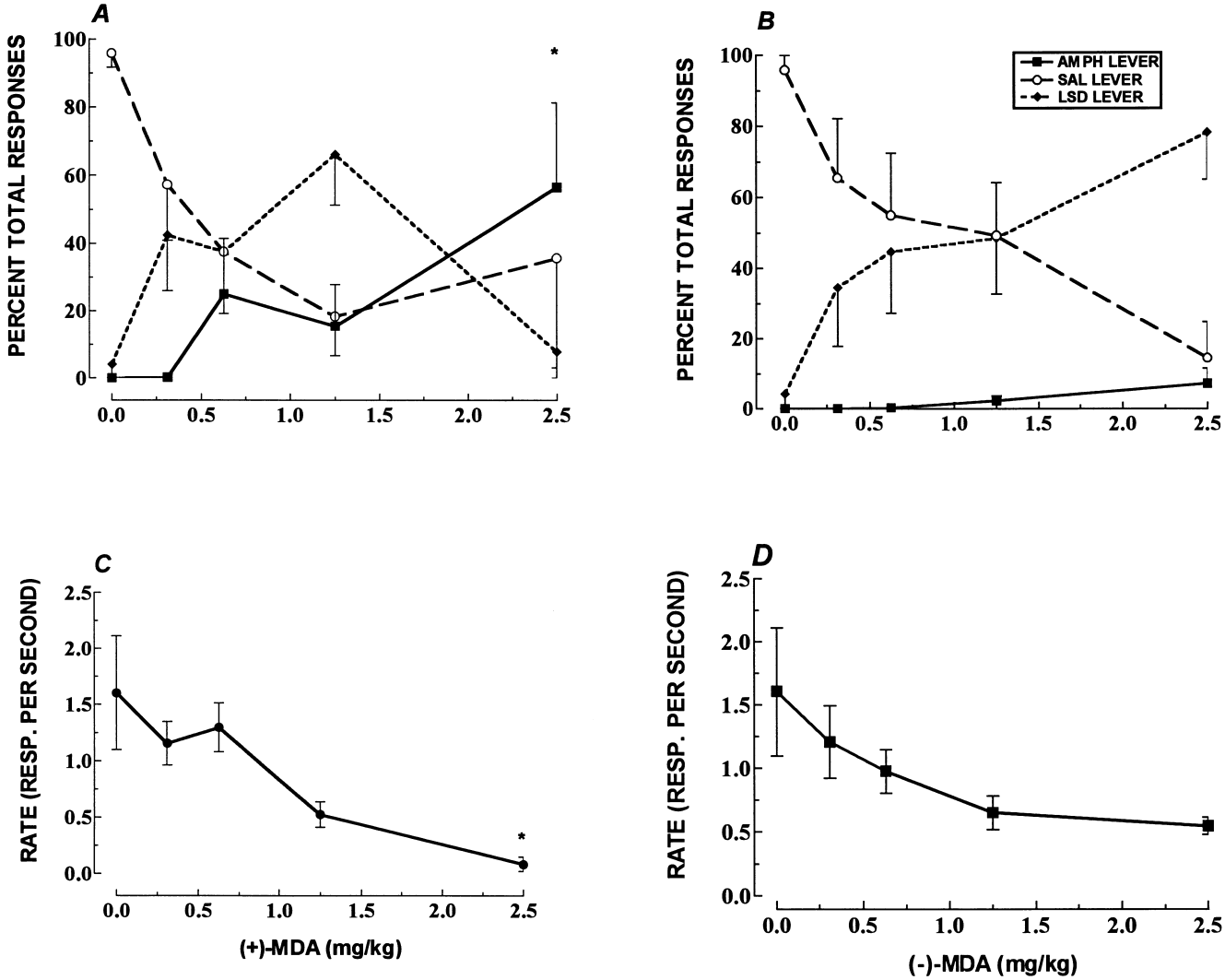

FIG. 5. Dose response functions for S(+)-MDA and R(-)-MDA. Graphs A and B depict percent total responses on each lever for S(+)-MDA and R(-)-MDA, repsectively. Graphs C and D show response rate for S(+)-MDA and R(-)-MDA, respectively. The * indicates doses that were behaviorally disruptive. (n = 8 at doses 0.312–1.25 mg/kg of both isomers; n = 2 at 2.5 mg/kg S(+)-MDA; n = 5 at 2.5 mg/kg R(-)-MDA)

nation was successfully trained in these subjects. Results of stimulus substitution tests with the optical isomers of MDMA and MDA confirm the results of experiment one, that these substances are qualitatively different from S(+)-amphetamine. Although a few animals generalized to S(+)-amphetamine when tested with the S(+)-isomers, very few S(+)-amphetamine-lever responses were emitted during tests with the R(-)-isomers. Whether higher doses of these isomers are more similar to S(+)-amphetamine was difficult to assess in these subjects because such doses were behaviorally disruptive. Only two animals emitted at least 20 responses when tested with 2.5 mg/kg S(+)-MDA, while the other three emitted only one or two responses. However four animals completed substitution tests with 2.5 mg/kg S(+)-MDMA, and the percent S(+)-amphetamine-lever and percent LSD-lever responses were equivalent at this dose.

Clearly, the extent to which the stereoisomers are hallucinogen- or stimulant-like depends on what drugs are initially established as discriminative stimuli. Although other investigators have reported that S(+)-MDA and S(+)-MDMA substitute for S(+)-amphetamine (7, 9) in rats trained to discriminate S(+)-amphetamine from saline, when rats are trained to discriminate either isomer of MDA (2) or MDMA (1) from saline, they do not generalize completely to the effects of S(+)-amphetamine. The present results indicate that when rats are trained to discriminate both S(+)-amphetamine and LSD, both isomers of MDA and MDMA produce greater responding on the LSD-lever. The greatest amount of LSD-appropriate responding was observed with R(-)-MDA. In fact, four of the five rats tested with 2.5 mg/kg R(-)-MDA completely generalized to LSD. These results are consistent with previous reports that R(-)-MDA substitutes for LSD (3) and LSD substitutes for R(-)-MDA (2). Unlike the highest dose of S(+)-MDA, S(+)-MDMA and R(-)MDMA, R(-)-MDA (2.5 mg/kg) was not behaviorally disruptive and produced nearly complete generalization to the LSD-appropriate lever.

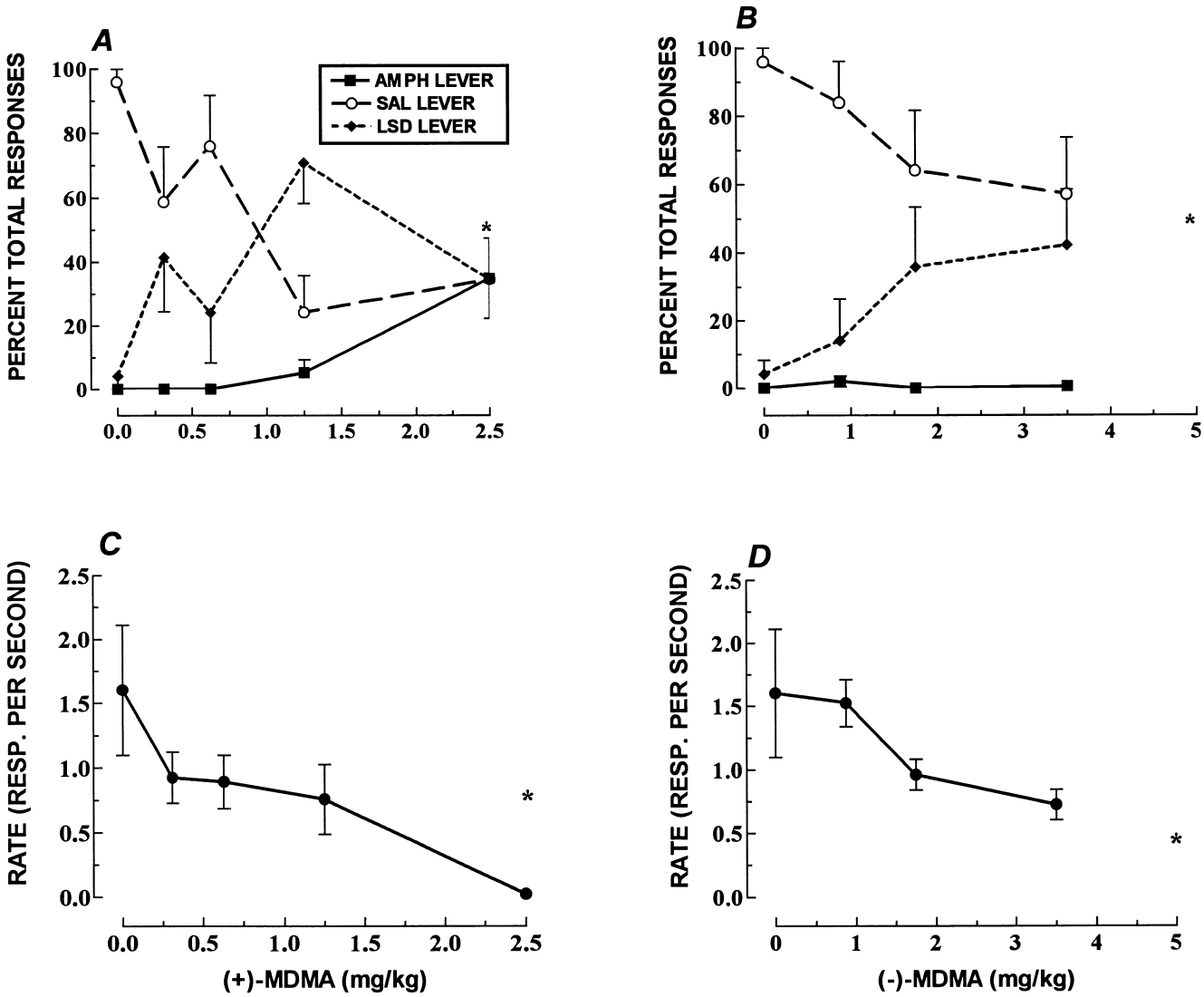

FIG. 6. Dose response functions for S(+)-MDMA and R(-)-MDMA. Graphs A and B depict percent total responses on each lever for S(+)-MDMA and R(-)-MDMA, repsectively. Graphs C and D show response rate for S(+)-MDMA and R(-)-MDMA, respectively. The * indicates doses that were behaviorally disruptive. (n = 8 at doses 0.312-1.25 mg/kg S(+)-MDMA and 0.875- 3.5 mg/kg R(-)MDMA; n = 4 at 2.5 mg/kg S(+)-MDMA, n = 0 at 5.0 mg/kg R(-)-MDMA)

Only one subject failed to exhibit generalization. Unfortunately, only five of the eight subjects were tested on the highest dose of each isomer. The remaining three subjects did not exhibit sufficient discriminative stimulus control by the end of the study when these doses were tested.

CONCLUSIONS

The results of both experiments demonstrated that rats can learn to discriminate a hallucinogen (mescaline or LSD) from S(+)-amphetamine in a three-choice drug discrimination situation. In contrast to previous reports from two-lever amphetamine discrimination investigations (7, 9), the S(+)-isomers of MDA and MDMA do not appear to mimic the discriminative stimulus effects of S(+)-amphetamine when the same subjects are also trained to discriminate either mescaline or LSD. Moreover, the present results indicate that when animals are trained to discriminate multiple drug stimuli, drugs that share similarities with both components may not produce complete generalization to either component. This appears to be the case with S(+)-MDA and S(+)-MDMA, which produced partial generalization to both LSD and S(+)-amphetamine (experiment two). However, the R(-)-isomers of these compounds appear to be more hallucinogenic than S(+)-amphetamine-like. Although, the R(-)-isomers did not substitute completely for mescaline at doses that were reported to substitute for mescaline in a two-choice drug discrimination (3). The closest approximation to stimulus generalization occurred with R(-)-MDA which produced nearly complete substitution for mescaline (79%) and LSD (78%). These results are consistent with previous reports (2, 3, 8).

One possible interpretation of the present study is that the discrimination established between S(+)-amphetamine and LSD is essentially a discrimination between dopamine-medi-

ated effects and serotonin-mediated effects. Partial responding on both drug levers would indicate that both serotonergic and dopaminergic effects are key components in the discriminable effect of the MDA and MDMA isomers. Greater substitution with R(-)-MDA for LSD could be interpreted to indicate that the serotonin release is a more salient component of this drug's discriminable effects. While both isomers of MDA and MDMA are approximately equipotent in their ability to facilitate serotonin release, the S(+)-isomers are more potent DA releasers (14). This could account for the greater amount of amphetamine-lever responses with the S(+)-isomers. Also, R(-)-MDA does have a higher affinity for 5-HT_2 receptors than S(3+)-MDA (313), which might account for the greater percentage of LSD-lever responding with R(-)-MDA.

An extensive review of the literature has revealed only two other three-choice drug discrimination studies that assessed the discriminative stimulus effects of MDA or MDMA. Young and Glennon (25) recently demonstrated that rats could be trained to discriminate the optical isomers of MDA. In addition, they reported that S(+)-amphetamine produced S(+)-MDA-appropriate responding and DOM produced R(-)-MDA-appropriate responding. However, this study is published only in abstract form and has not been replicated. The only other published three-choice drug discrimination procedure in which MDMA and MDA were assessed was a study conducted in pigeons in which S(+)-amphetamine and fenfluramine were used as training drugs (35). The racemic mixtures of MDMA as well as both S(+)- and R(-)-MDA were found to produce a mixture of fenfluramine and S(+)-amphetamine-appropriate responses and the results among individual subjects were highly variable. Since fenfluramine and S(+)-amphetamine possess distinct stimulus properties, the findings from Evans et al. (35) further support the notion that MDMA and MDA possess compound discriminative stimulus properties.

The present results are also interesting in light of reports that MDMA and MDA produce subjective experiences similar to, yet uniquely distinct from both stimulants and hallucinogens in humans (17). Some investigators have suggested these drugs may represent a novel therapeutic drug class (e.g., "entactogens", (15). Since reports of LSD-like hallucinations with MDMA in humans is uncommon, the present results that the isomers of both MDA and MDMA appear more like LSD than amphetamine may be questioned. However, to our knowledge, there are no published data on the subjective effects of the individual isomers of MDMA or MDA in humans. Thus, direct comparisons between the present data and human subjective reports may not be made.

In summary, rats can learn to discriminate both hallucinogens and stimulants using a three-choice drug discrimination procedure. Stimulus substitution tests with the optical isomers of MDA and MDMA in rats trained in this manner indicate that these drugs may not be as similar to amphetamine as suggested in previous reports based on two-choice drug discrimination experiments. The present results do support previous reports that MDMA and MDA possess complex stimulus properties (2, 5, 20, 31). Moreover, the degree of similarity between these compounds and either stimulants or hallucinogens clearly depends on the training drug initially used to establish discriminative stimulus control. Although the three-choice drug discrimination procedure is extremely time consuming, it may provide a greater degree of precision in which to investigate the complex discriminative stimulus properties of some compounds than the more traditional two-choice discrimination procedure.

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