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Discriminative stimulus effects of the optical isomers of 3,4-methylenedioxyamphetamine (MDA)

J. Broadbent, J.B. Appel, E.K. Michael and J.H. Ricker

Behavioral Pharmacology Laboratory, Department of Psychology, University of South Carolina, Columbia, SC 29208, USA

Correspondence to: J. Broadbent, Medical Psychology, L470, Oregon Health Sciences University, 3181 SW Sam Jackson Park Road, Portland, OR 97201-3098, USA

The isomers of 3,4-methylenedioxyamphetamine (MDA) functioned as discriminative stimuli in rats trained to discriminate either (-) MDA (1.25 mg/kg) or (+) MDA (1.25 mg/kg) from saline. Dose- and time-response curves indicated that drug lever selection occurred at doses of at least 1.00 mg/kg of (-) MDA and 0.75 mg/kg of (+) MDA and that drug-appropriate responding for both isomers was maintained for at least 90 min. Cross-substitution was observed between the MDA isomers; both (+) and (-) 3,4-methylenedioxymethamphetamine (MDMA) also substituted completely for (+) and (-) MDA. The hallucinogens (+)-lysergic acid diethylamide (LSD) and (±)-2,5-dimethoxy-4-methylamphetamine (DOM), substituted for (-) MDA; neither mescaline nor (+) amphetamine or cocaine had (-) MDA-like effects. LSD also substituted for (+) MDA; DOM, mescaline, (+) amphetamine and cocaine failed to have (+) MDA-like effects. The (-) but not the (+) MDA cue was blocked by the 5-HT$_2$ antagonist pirenpirone; the dopamine (DA) antagonists SCH-23390 and (-) sulpiride had no effect on either the (-) or (+) MDA cues. When animals were trained to discriminate LSD (0.16 mg/kg) or (+) amphetamine (1.0 mg/kg) from saline, neither (-) MDA nor (+) MDA substituted completely. These results indicate that: (1) the stimulus effects of the isomers of MDA and MDMA are similar; (2) (-) MDA may be more hallucinogenic (or more accurately, LSD- or DOM-like) than (+) MDA; (3) neither (+) nor (-) MDA has potent amphetamine-like effects; and (4) the effects of (-) MDA may be more serotonergic than those of (+) MDA.

Keywords: (+) Amphetamine – Cocaine – DOM – Drug discrimination – LSD – MDA – MDMA – Mescaline – Rat

INTRODUCTION

Due to their supposedly unique subjective effects, which are said to include the ability to enhance communication and empathy, substances such as 3,4-methylenedioxyamphetamine (MDA, "love") and 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") have been used recreationally (Weil, 1976; Downing, 1986; Peroutka, 1987) and promoted as adjuncts to psychotherapy (Jackson and Reed, 1970; Yensen *et al.*, 1976; Barnes, 1988). However, recent reports that these "designer drugs" have toxic effects on serotonin (5-HT) neurons (Battaglia *et al.*, 1987; Schmidt, 1987; Stone *et al.*, 1987; Johnson *et al.*, 1988; Insel *et al.*, 1989; Gibb *et al.*, 1990; Schmidt and Taylor, 1990) amplify the need to delineate further the behavioral neuropharmacology of what has been said to constitute a new class of psychoactive compounds, *entactogens* (Nichols and Oberlender, 1990).

Although phenylalkylamines such as MDA and MDMA structurally resemble both (+) amphetamine (phenylisopropylamine) and mescaline (3,4,5- trimethoxyphenylethylamine), purported similarities between these designer drugs and both stimulants and hallucinogens (Nozaki *et al.*, 1977; Marquardt *et al.*, 1978) are surprising in that the neuronal mechanisms underlying the *in vivo* effects of these substances are quite different (amphetamines are primarily catecholaminergic while hallucinogens are serotonergic). Nevertheless, drug discrimination experiments support the idea that MDA has both stimulant- and hallucinogen-like properties in that racemic MDA has been found to mimic (+) amphetamine as well as (±)-2,5-dimethoxy-4-methylamphetamine (DOM) (Glennon and Young, 1984a, b). Interestingly, tests with the individual isomers of MDA in animals trained to discriminate either a hallucinogen (DOM) or a stimulant ((+) amphetamine) from saline, indicate that these isomers have dissimilar subjective effects: (+) MDA mimics (+) amphetamine but not DOM while (-) MDA mimics DOM but not (+) amphetamine (Glennon *et al.*, 1982; Glennon and Young, 1984c).

Nevertheless, the individual isomers of MDA require further investigation because recent results raise additional questions about their subjective effects. For example, since both enantiomers of MDA (as well as MDMA) have been reported to substitute for the mescaline cue (10 mg/kg), the two isomers of these substances may not be completely dissimilar (Callahan and Appel, 1988); in addition, MDA does not necessarily have (+) amphetamine-like effects (Shannon, 1980; Oberlender and Nichols, 1990). Thus, the present study attempted to clarify the stimulus properties of (+) and (−) MDA by training animals to discriminate both of these substances from saline and examining: (1) the extent to which the isomers of both MDA and MDMA substitute for one other; (2) the ability of a number of prototypic stimulants and hallucinogens to substitute for comparable doses of each isomer; and (3) the effects of selective 5-HT_2 and DA antagonists on both the (+) and (−) MDA cues. Further, to assess the symmetry of cross-substitution under similar experimental conditions between the two isomers of MDA and both central nervous system stimulants and hallucinogens, two additional groups of animals were trained to discriminate (+) amphetamine (1 mg/kg) and (+) LSD (0.16 mg/kg) from saline, and tested with both (+) and (−) MDA.

METHODS

Subjects

Experimentally-naive male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 350-450 g were used. Animals were housed individually with food and water freely available. Following a 2-week habituation period, access to water was restricted to that available during training sessions, 10-15 min in home cages after test sessions, and 24-46 h on weekends. The colony was maintained at 22°C (± 1); lights were on from 0.700 h to 19.00 h.

Apparatus

Training and testing sessions were conducted in 16 commercially-available chambers (BRS/LVE Model No. 143-24), each of which was furnished with a house light and a dipper mounted equidistant between two levers. Sound- and light-attenuating shells (BRS/LVE Model No. 132-04) equipped with fans, provided both ventilation and masking noise. All experimental events and data collection were controlled by Apple IIe microcomputers located in an adjoining room.

Procedure

Shaping. Following a period of water deprivation, separate groups of animals were trained to discriminate i.p. injections of (+) MDA (1.25 mg/kg), (−) MDA (1.25 mg/kg), (+) LSD (0.16 mg/kg), or (+) amphetamine (1 mg/kg), from i.p. injections of 1 ml/kg of isotonic saline (vehicle). Fifteen minutes later, they were placed in the experimental chambers and trained to press a lever for water (0.1 ml) under a fixed ratio (FR) 1 schedule; as response rate stabilized the FR was increased gradually to a final value of FR 20. Only the stimulus-appropriate lever was present during shaping sessions ("errorless" training), which were 20 min in duration and occurred at the same time of day, 5 or 6 days per week. Drug and saline sessions were given in random order with the restriction that no condition occur for more than 3 days in succession. Within each of the training groups, the drug-appropriate lever was designated as the right lever for half of the animals and left for the remaining subjects.

Discrimination training. Once consistent responding under the FR 20 schedule occurred, the drug- and saline-appropriate levers were presented simultaneously. Reinforcement was contingent on completion of the FR on the stimulus-appropriate lever; responding on the stimulus-inappropriate lever was recorded but had no consequences. To avoid the presence of olfactory cues (Extance and Goudie, 1981), the levers were wiped with alcohol before each 20-min session. Animals reaching a criterion of a mean of 80% stimulus-appropriate responding during the completion of the first FR on 10 consecutive training sessions were tested with novel compounds.

Testing. Novel compounds were administered before 20-min extinction test sessions; that is, completion of 20 responses on either lever or the passing of 20 min terminated the test without reinforcement. All animals received doses of test drugs in a mixed order. In addition, the accuracy of discrimination was assessed periodically in test sessions following either saline or training drug injections. Test sessions were conducted once or twice per week on different days of the week providing the animals maintained an accuracy of 80% on the three preceding training days.

Drugs

The following drugs were dissolved in saline and administered i.p. in a volume of 1 ml/kg, 15 min before training or test sessions: (−) MDA hydrochloride, (+) MDA hydrochloride, (+) LSD bitartrate, (±) DOM hydrochloride (National Institute on Drug Abuse, NIDA; Rockville, MD), (+) amphetamine sulfate, cocaine hydrochloride, mescaline hydrochloride (Sigma Chemical Co., St Louis, MO). Pirenpirone (Janssen Pharmaceutica, New Brunswick, NJ), (−) sulpiride (Research Biochemicals Inc., Natick, MA, dissolved in a minimum of 3 N glacial acetic acid and brought up to volume with saline), and SCH-23390 maleate (Schering Corp., Bloomfield, NJ)

were injected at 60 (i.p.), 150 (s.c.) and 30 (s.c.) min respectively before the start of test sessions. Antagonists were injected in addition to the training dose of MDA. Vehicle solutions, given either i.p. or s.c. at appropriate times before the training drug, were used as controls in antagonist tests. Doses of pirenpirone and (−) sulpiride refer to the free bases; all other doses refer to the salts. Appropriate doses and routes of administration of test compounds were chosen based on previous reports from the literature.

Data Analysis

The percentage drug-appropriate responding was calculated from training and test sessions by dividing the number of responses on the drug-appropriate lever by the total number of responses emitted to completion of the first 20 responses (× 100) on one or the other lever. Rates of responding were defined as the total number of responses emitted to completion of 20 responses on either lever, divided by the number of minutes to the completion of 20 responses on either lever. Data from animals not completing 20 responses on either lever were discarded. ED_{50} values and 95% confidence limits were calculated according to the methods of Litchfield and Wilcoxon (1949).

The data from animals completing the FR were compared to training drug controls using repeated measures analyses of variance (ANOVA) followed by comparison of test doses with drug control using Dunn's (Bonferroni *t*) procedure (Keppel, 1982) when significant differences were detected in ANOVA tests. Data from animals not completing the FR at a test dose were not included in the ANOVA at that dose. ANOVAs with unequal cell sizes were calculated using the method of unweighted means (Kirk, 1982). Complete substitution was said to occur when responding reached a mean of at least 80% on the drug-appropriate lever, and, responding did not differ significantly from training drug control (one-tail $t, p > 0.05$). Similarly, complete antagonism was said to occur when test doses were significantly different from training drug control, and, significant differences were not observed between test doses and vehicle control. Response rates were also compared with test drug controls using repeated measures ANOVAs followed by Dunn's (Bonferroni *t*) procedure (two-tail $t, p < 0.05$). Doses at which fewer than five subjects completed the FR were not subjected to statistical analyses.

RESULTS

The rats learned to discriminate each of the training drugs from saline and maintained criterion levels of accuracy (at least 80% correct) throughout the experiment (data not shown). All subjects trained on (−) and (+) MDA acquired the discrimination (n = 16/isomer): subjects trained on (−) and (+) MDA required a mean (± S.E.M.) of 25 (± 3) and 16 (± 1) sessions, respectively, to reach criteria for testing (see Methods).

Since the parameters of the discrimination of the individual isomers of MDA from saline have not been reported previously, both dose- and time-response curves are shown in Fig. 1. Analysis of the data from animals trained to discriminate (−) MDA (1.25 mg/kg) indicate that doses of (−) MDA below 1.0 mg/kg did not consistently engender drug-appropriate responding (Fig. 1, left panel). The ED_{50} for (−) MDA [95% confidence limits (C.L.)] was 0.50 (0.29-0.88) mg/kg. Comparison of response rates with (−) MDA (1.25 mg/kg) controls did not reveal significant differences [$F(4,20) = 2.2, p > 0.05$]. When the injection-test intervals were lengthened beyond the training-test interval (15 min), responding at levels exceeding 80% occurred on the drug-appropriate lever for up to 90 min following i.p. injection, but not after 120 min ($p < 0.05$, Fig. 1). Subjects tested at a different pre-treatment interval completed the tests with a mean (± S.E.M.) latency of 101 (± 11) s from the start of the test to the completion of the FR. Response rate did not differ significantly from 15 min drug controls [$F(4,28) = 0.65, p > 0.05$].

Decreasing doses in animals trained to discriminate 1.25 mg/kg of (+) MDA from saline (Fig. 1, right panels) produced a relatively steep dose-response curve; responding on the drug-appropriate lever exceeded 80% at doses greater than 0.5 mg/kg. The ED_{50} value (95% C.L.) for (+) MDA was 0.54 (0.48-0.61) mg/kg. The response rate of subjects administered 0.25 mg/kg (+) MDA differed from (+) MDA (1.25 mg/kg) controls ($p < 0.05$). As was the case with the (−) isomer, high levels of drug-appropriate responding persisted for at least 90 min. Again, subjects tested at the different pre-treatment intervals completed the tests rapidly with a mean (± S.E.M.) latency of 63 (± 8) s. Although analysis of variance revealed a tendency toward higher response rates across the time intervals tested when compared with (+) MDA given at 15 min [$F(2,24) = 3.56, p < 0.05$], *post hoc* tests did not indicate significant differences at the individual doses ($p > 0.05$).

The results of substitution tests with the isomers of both MDA and MDMA in animals trained to discriminate (+) or (−) MDA from saline are shown in Table I. At a dose of 1.25 mg/kg, (−) MDA did not substitute for 1.25 mg/kg of (+) MDA ($p < 0.05$) while 1.5 mg/kg substituted completely; (+) MDMA substituted completely for (+) MDA at a dose of 1.25 mg/kg while (−) MDMA only substituted for (+) MDA at doses as high as 2.5 and 3.0 mg/kg [$F(2,28) = 1.94, p > 0.05$]. Analyses of response rates following administration of (−) MDA, (+) MDMA and (−) MDMA did not suggest differences from (+) MDA controls [$F(2,28) = 2.19$, $F(2,26) = 0.53$, and $F(2,28) = 1.1, p > 0.05$ respectively]. Doses of (+) MDA (1.25 mg/

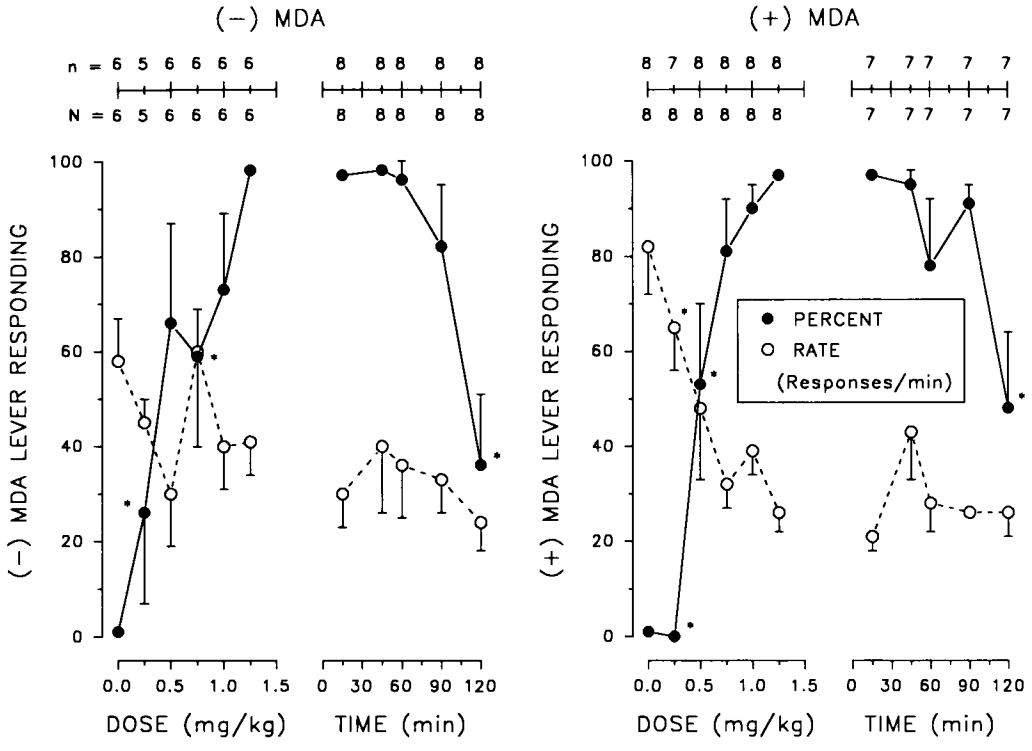

FIG. 1. Dose- and time-response curves in rats trained to discriminate 1.25 mg/kg of (−) MDA (left panels) or 1.25 mg/kg of (+) MDA (right panels) from saline. Closed circles denote the mean percentage responding on the lever associated with the training drug (± S.E.M.) during test sessions: open circles denote mean (± S.E.M.) response rates (responses/min). The numbers at the top of each graph indicate the number of animals completing 20 responses on one or the other lever (*n*) and the number of animals tested (*N*) at each dose, including control vehicle (0) or injection-test interval (15 min). Asterisks represent doses where responding differed significantly from training drug controls ($p < 0.05$).

kg), (+) MDMA (1.25 mg/kg), and (−) MDMA (3.0 mg/kg) completely substituted for the (−) MDA (1.25 mg/kg) cue ($p > 0.05$). Both (+) MDMA and (−) MDMA suppressed response rates relative to those of (−) MDA controls ($p < 0.05$).

The results of substitution tests with drugs that act primarily through 5-HT neuronal systems, in rats trained to discriminate (−) MDA (1.25 mg/kg) from saline are shown in Fig. 2. The indolealkylamine hallucinogen LSD and the phenylethylamine hallucinogen DOM substituted completely for (−) MDA; ED_{50} values (95% C.L.) for LSD and DOM respectively were 0.013 (0.007-0.024) mg/kg and 0.12 (0.06-0.23) mg/kg. These substitutions were confirmed by the fact that drug-appropriate responding following 1.25 mg/kg of (−) MDA and LSD (0.04-0.1 mg/kg) were not significantly different [$F(4,44) = 2.2, p > 0.05$]; a sufficiently high dose of DOM (1.0 mg/kg) also substituted completely for (−) MDA ($p > 0.05$). Response rates following administration of LSD or DOM were significantly different from (−) MDA controls [mean response rate (± S.E.M.) of 34 (± 6), $F(4,44) = 5.56$, and $F(4,44) = 6.71, p < 0.05$ respectively]. The phenylethylamine hallucinogen mescaline did not engender (−) MDA-like responding at the doses tested [ED_{50} (95% C.L.) = 10.4 (8.36-12.94) mg/kg]. Further, response rates were not significantly decreased by administration of mescaline when compared with (−) MDA controls [$F(3,39) = 2.42, p > 0.05$]. At a higher dose (15 mg/kg; data not shown), mescaline disrupted responding in 10 of the 14 subjects tested and engendered only 44 (± 1)% drug-appropriate responding and a response rate of 28 (± 19) responses/min.

Tests with prototypic stimulants in animals trained to discriminate (−) MDA from saline (Fig. 3), did not indicate similarities between the stimulus properties of either (+) amphetamine or cocaine and those of (−) MDA [ED_{50} (95% C.L.) of 25.1 (4.58-137.7) mg/kg and 39.8 (23.1-68.7) mg/kg respectively]. Response rates following amphetamine injection did not differ from (−) MDA response rates of 30 ± 5 [$F(3,39) = 1.70, p > 0.05$]. A higher dose of (+) amphetamine (1.25 mg/kg, data not shown) produced a mean (± S.E.M.) of 31 (± 16)% (−) MDA-appropriate responding with a response rate of 11 (±4) responses/min in the six animals completing the test:

TABLE I. Results of substitution tests[1] with the isomers of MDA and MDMA

Training drug	Test drug	Dose (mg/kg)	Percentage[2] (± S.E.M.)	Rate[3] (± S.E.M.)	*n*/*N*[4]
(+) MDA		1.25	98 ± 1	27 ± 3	15/15
	(–) MDA	1.25	49 ± 11*	42 ± 6	15/15
		1.50	92 ± 4	46 ± 11	15/15
	(+) MDMA	1.00	76 ± 11	35 ± 7	14/14
		1.25	95 ± 2	30 ± 7	14/14
	(–) MDMA	2.50	84 ± 3	31 ± 5	15/15
(–) MDA		1.25	97 ± 1	31 ± 5	16/16
	(+) MDA	1.25	97 ± 2	25 ± 7	16/16
	(+) MDMA	1.25	100 ± 0	16 ± 4*	15/15
	(–) MDMA	3.00	96 ± 2	17 ± 3*	15/15

[1] All tests were conducted 15 min after i.p. injection.
[2] Percentage of total number of responses during each test trial occurring on the drug-appropriate lever.
[3] Responses/min.
[4] *n* = number of animals completing 20 responses; *N* = number of animals tested.
*$p < 0.05$ from training drug controls.

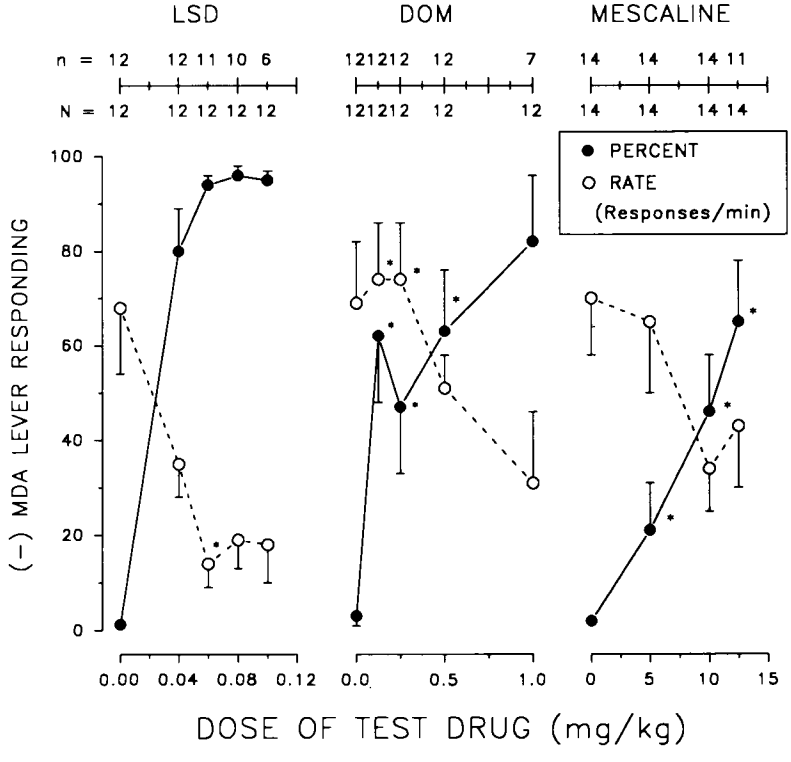

FIG. 2. Results of substitution tests with hallucinogenic compounds in animals trained to discriminate (–) MDA (1.25 mg/kg) from saline. All drugs were given i.p., 15 min prior to behavioral testing. Closed circles denote the mean percentage responding on the lever associated with the training drug (± S.E.M.) during test sessions: open circles denote mean (± S.E.M.) response rates (responses/min). The numbers at the top of each graph indicate the number of animals completing 20 responses one on or the other lever (*n*) and the number of animals tested (*N*) at each dose, including control vehicle. Asterisks represent doses where responding differed significantly from training drug controls ($p < 0.05$).

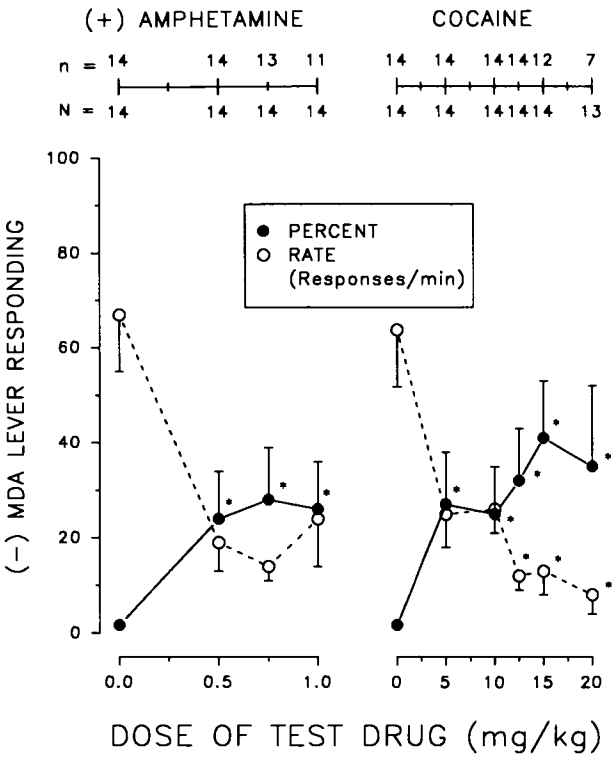

FIG. 3. Results of substitution tests with CNS stimulants in animals trained to discriminate (–) MDA (1.25 mg/kg) from saline. All drugs were given i.p., 15 min before behavioral testing. See Fig. 2 for further details.

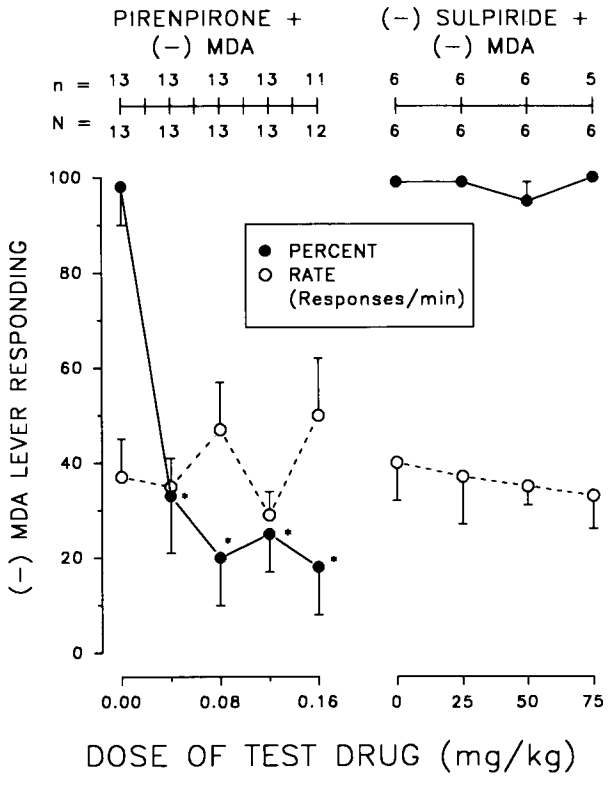

FIG. 4. Results of combination tests with 5-HT and DA antagonists in animals trained to discriminate (–) MDA from saline. Pirenpirone was given i.p., 60 min prior to testing (45 min prior to MDA); (–) sulpiride was given s.c., 150 min prior to testing (135 min prior to MDA). Controls at 0 on the horizontal axis are vehicle plus (–) MDA (1.25 mg/kg). See Fig. 2 for further details.

responding was disrupted in an additional eight animals. Administration of cocaine significantly decreased response rates when compared with (–) MDA controls responding at 30 (± 3) responses/min [$F(5,65) = 3.45, p < 0.05$].

The (–) MDA cue was completely antagonized by the 5-HT_2 antagonist pirenpirone (Fig. 4); ANOVA indicated significant differences between test [antagonist + (–) MDA doses] and (–) MDA controls ($F(4,44) = 18.54, p < 0.05$), but not vehicle controls [$F(4,48) = 2.14, p > 0.05$; AD_{50} (95% C.L.) = 0.011 (0.002-0.071) mg/kg]. Response rates did not differ significantly from (–) MDA controls [saline + (–) MDA, mean (± S.E.M.) of 37 (± 8) responses/min, $F(4,48) = 1.3, p > 0.05$]. The D_2 antagonist (–) sulpiride did not alter drug-appropriate responding [$F(3,15) = 1.06, p > 0.05$] or response rates [$F(3,15) = 0.1, p > 0.05$] from (–) MDA controls. Unfortunately, disruption of behavior by low doses of SCH-23390 (0.05 and 0.1 mg/kg) in combination with (–) MDA (data not shown), precluded testing of this compound in a sufficiently large number of animals to produce meaningful data.

The results of parallel tests in rats trained to discriminate (+) MDA (1.25 mg/kg) from saline are shown in Figs 5-7. LSD substituted for this isomer, but DOM, mescaline, cocaine, and (+) amphetamine did not. More specifically, LSD produced dose-related increases in (+) MDA-appropriate responding (to a maximum of 80% at 0.08 mg/kg) which did not differ significantly from training drug controls at 0.08 and 1.0 mg/kg [$p > 0.05$: ED_{50} (95% C.L.) = 0.052 (0.04-0.06) mg/kg]. Response rates at the doses of LSD tested were similar to those of (+) MDA controls [mean (± S.E.M.) of 40 (± 8) responses/min, $F(4,36) = 1.04, p > 0.05$]. DOM did not substitute for (+) MDA [ED_{50} (95% C.L.) = 0.83 (0.65-1.06) mg/kg]. Although drug-appropriate responding following the highest dose of DOM (1.25 mg/kg; Fig. 5), failed to differ significantly from (+) MDA controls ($p = 0.052$), only 65% of responses were on the drug-appropriate lever and therefore failed to reach criterion for substitution (see Methods). Mescaline also failed to substitute for the training drug [ED_{50} (95% C.L.) = 17.0 (8.9-32.4) mg/kg]. While rate of responding following a low dose of DOM (0.125 mg/kg) was elevated in relation to (+) MDA controls [mean (± S.E.M.) of 26 (± 5) responses/min, $F(5,40) = 4.79, p < 0.05$], response rates after higher doses did not differ from

drug controls ($p > 0.05$). Similarly, response rates at a low dose of mescaline (5 mg/kg) were significantly higher than (+) MDA controls [mean (± S.E.M.) of 27 (± 4), $F(4,52) = 11.38$, $p < 0.05$] while a higher dose (12.5 mg/kg) decreased response rates below those of drug controls ($p < 0.05$).

Amphetamine did not fully substitute for (+) MDA at any dose tested, engendering a maximum of 60% drug-appropriate responding [Fig. 6; ED_{50} (95% C.L.) = 0.83 (0.6-1.15) mg/kg]; at a higher dose of 1.5 mg/kg (data not shown), only 2 of the 15 subjects tested completed the FR, 48% of total responses occurring on the drug-appropriate lever at a rate of 4 responses/min. Administration of (+) amphetamine did not alter response rates from those of (+) MDA controls [mean (± S.E.M.) of 27 (± 3) responses/min, $F(4,56) = 2.37$, $p > 0.05$]. Although more drug-appropriate responding occurred following treatment with cocaine (maximum of 75%, Fig. 6), the amount of drug-lever responding at 20 mg/kg was significantly different from that induced by the training drug [$p = 0.049$, ED_{50} (95% C.L.) = 13.5 (4.7-38.6) mg/kg]. Response rate was reduced below that of (+) MDA controls [mean (± S.E.M.) of 27 (± 3) responses/min, $F(5,70) = 3.81$, $p < 0.05$] by only one dose of cocaine (15 mg/kg). Tests with 22.5 mg/kg of cocaine (data not shown) did not result in increased responding on the (+) MDA-appropriate lever [mean (±S.E.M.) of 60 (± 17)% at 25 (± 9) responses/min] and disrupted responding in 8 of the 15 animals tested.

The 5-HT_2 antagonist pirenpirone (0.04-0.16 mg/kg) and the dopamine antagonists SCH-23390 (0.1-0.3 mg/kg), and (−) sulpiride (25-100 mg/kg), when injected in combination with (+) MDA, failed to alter the amount of drug-appropriate responding (data not shown). Lever selection and response rate following administration of pirenpirone in combination with (+) MDA (1.25 mg/kg) did not differ significantly from those after injection of (+) MDA alone in the 12 subjects tested [$F(4,44) = 2.39$, $F(4,44) = 1.98$, $p > 0.05$ respectively]. The effects of SCH-23390 and (−) sulpiride could not be assessed statistically since fewer than five subjects completed 20 responses at most doses during test sessions.

Finally, the results of substitution tests with the isomers of MDA in rats trained to discriminate either (+) amphetamine (1.0 mg/kg) or LSD (0.16 mg/kg) from saline are shown in Fig. 7. Subjects trained on (+) amphetamine or LSD displayed mean (± S.E.M.) drug-appropriate responding of 99 (± 1)% and 98 (± 1)%, respectively, and response rates of 17 (± 4) and 15 (± 3) responses/min respectively, during training drug control tests. Neither

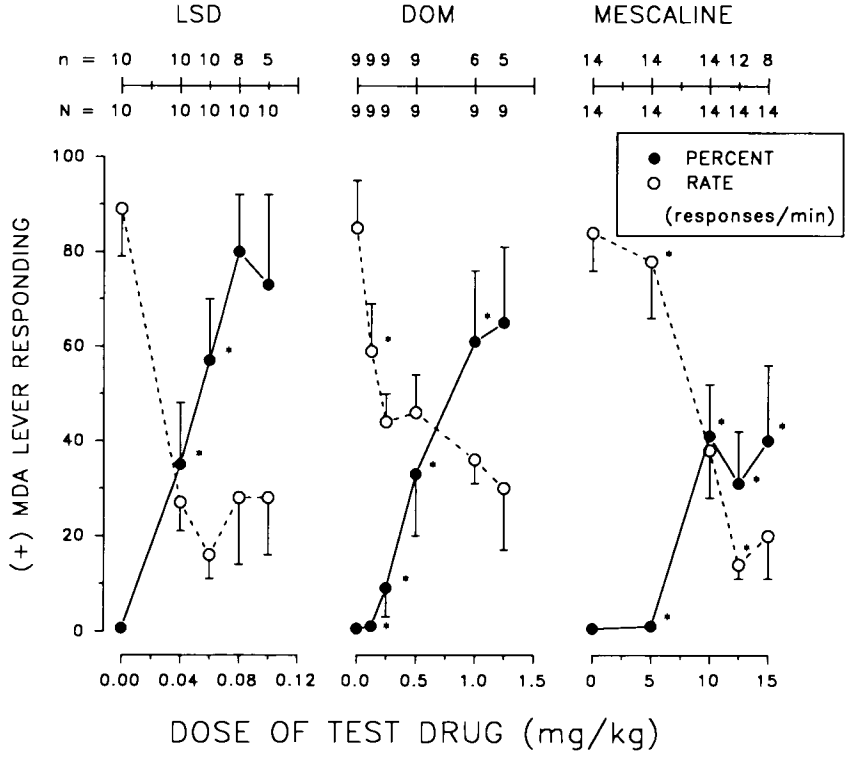

FIG. 5. Results of substitution tests with hallucinogenic compounds in animals trained to discriminate (+) MDA (1.25 mg/kg) from saline. All drugs were given i.p., 15 min before behavioral testing. See Fig. 2 for further details.

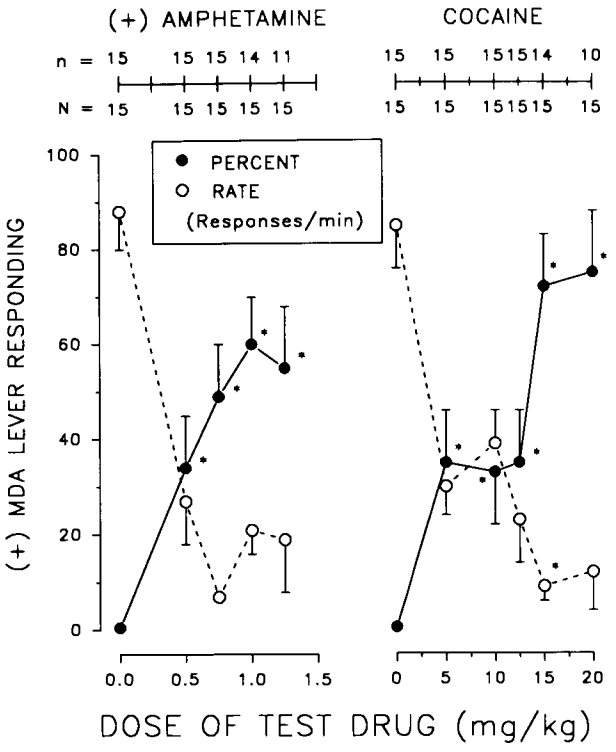

FIG. 6. Results of substitution tests with CNS stimulants in animals trained to discriminate (+) MDA (1.25 mg/kg) from saline. All drugs were given i.p., 15 min before behavioral testing. See Fig. 2 for further details.

isomer of MDA substituted for (+) amphetamine [Fig. 7, left panels; ED_{50} (95% C.L.) for (+) and (−) MDA, respectively, were 1.77 (1.0-3.2) mg/kg and 26.2 (2.1-327 mg/kg]. Due to the small sample size at several doses of (+) MDA, these data were not subjected to statistical analyses. Drug-appropriate responding following each dose of (−) MDA, however, differed significantly from (+) amphetamine controls ($p < 0.05$). While statistical analyses suggested response rates after (−) MDA administration tended to differ from (+) amphetamine controls [$F(4,28) = 2.99$, $p < 0.05$], response rates at individual doses did not differ significantly from control ($p > 0.05$). Tests with a higher dose of (−) MDA (1.75 mg/kg; data not shown) resulted in three of eight animals responding with a mean (± S.E.M.) of 29 (± 29)% amphetamine-appropriate responding and a response rate of 15 (± 5) responses/min.

Neither (+) nor (−) MDA substituted for the stimulus effects of LSD [Fig. 7, right panels; ED_{50} (95% C.L.) = 1.07 (0.77-1.47) mg/kg and 1.56 (1.41-1.71) mg/kg respectively]. Response rates following both (+) and (−) MDA were not significantly different from drug controls [$F(3,21) = 0.19$, and $F(3,39) = 1.36$, $p > 0.05$, respectively]. Higher doses of (+) (1.75 mg/kg) and (−) (2.25 mg/kg) MDA (data not shown) did not substitute for LSD with responding on the LSD-appropriate lever of 59 (± 14)% and 62 (± 31)% respectively. Response rates were 7 (± 3) responses/min at 1.75 mg/kg of (+) MDA and 21 (± 14) responses/min at 2.25 mg/kg of (−) MDA in the three animals completing the FR from a total of eight subjects tested.

DISCUSSION

The present study demonstrates (for the first time) that animals can learn to discriminate the individual isomers of MDA from saline, thus enabling the purportedly different stimulus effects of these substances to be analyzed directly. The (+) and (−) isomers of MDA appear to be similar in that their effects last for at least 90 min and the isomers substitute for one another. Further, although response rates in each group were decreased from vehicle controls, rates of responding following administration of the training dose of (+) or (−) MDA (1.25 mg/kg) did not differ significantly from one another. Their potencies did differ slightly, however; 1.5 mg/kg of (+) MDA is required to obtain complete substitution for 1.25 mg/kg of (+) MDA while 1.25 mg/kg of (+) MDA substitutes completely for (−) MDA (Table I). Similarly, both (+) and (−) MDMA substitute completely for both isomers of MDA. A difference in the potencies of the isomers of MDMA was observed, however, with approximately twice as much (−) MDMA (2.5-3.0 mg/kg) being required to obtain the same effect as (+) MDMA (1.25 mg/kg).

The present results, as well as previous findings from our own (Callahan and Appel, 1988) and other laboratories (Glennon *et al.*, 1982; Nichols *et al.*, 1986), suggest that the stimulus effects of LSD, DOM and (−) MDA are similar, regardless of the drug used as the training stimulus. Since indole- and phenylethylamine hallucinogens such as LSD and DOM are thought to act primarily through exciting post-synaptic 5-HT (5-HT_2) or 5-HT_{1C} receptors, it seems reasonable to conclude that (−) MDA also acts, at least in part, through 5-HT mechanisms. This hypothesis is strengthened by findings that the stimulus effects of both LSD (Colpaert and Janssen, 1983; Cunningham and Appel, 1987), and (−) MDA (Fig. 4), in addition to other behavioral effects of (−) MDA (Rosecrans and Glennon, 1987) are blocked by the 5-HT_2 antagonist pirenpirone but not by the DA antagonist (−) sulpiride [at doses which effectively block both the discriminative stimulus effects of (+) amphetamine (Nielsen and Jepsen, 1985) and lisuride (Cunningham *et al.*, 1987)].

The conclusion that the stimulus effects of (−) MDA are similar to those of hallucinogens might be questioned by the failure of mescaline to substitute completely for (−) MDA (Fig. 2) and the fact that (−) MDA did not substitute completely in LSD-trained animals (Fig. 7). However, (−) MDA substitutes for lower training doses of LSD

(Nichols, 1986; Callahan and Appel, 1988) and for mescaline when the hallucinogen is used as the training stimulus (Callahan and Appel, 1988). Discrepancies of this nature emphasize the importance of both training drug and dose in interpreting the results of drug discrimination experiments. For example, relatively high doses of challenge (test) drugs may be required for subjectively similar compounds to substitute for potent training drugs (Stolerman and D'Mello, 1981; White and Appel, 1982; Wood *et al.*, 1985), but the same high doses may cause so much behavioral disruption that further testing (which might result in complete substitution) is precluded.

The present results are consistent with previous findings that the (−) isomer of MDA does not have potent stimulant-like effects (Glennon and Young, 1984c; Glennon *et al.*, 1988; Oberlender and Nichols, 1988). Neither (+) amphetamine nor cocaine substituted completely for (−) MDA (Fig. 3) and (−) MDA did not substitute at all for the (+) amphetamine cue (Fig. 7). More importantly, the data suggest that the effects of (−) MDA and central nervous system stimulants involve different mechanisms, since DA antagonists such as SCH-23390 and (−) sulpiride, which had no effect on the stimulus properties of (−) MDA (Fig. 4), attenuate the stimulus effects of cocaine and (+) amphetamine (Nielsen and Jepsen, 1985; Kleven *et al.*, 1988).

At an equivalent training dose (1.25 mg/kg), the results with the (+) isomer of MDA are not as clear as those with the (−) isomer. Although LSD substituted for (+) MDA (Fig. 5), DOM failed to substitute completely. These data, in addition to the failure of (+) MDA to substitute for LSD despite the use of two different training doses (Fig. 7; Nichols *et al.*, 1986; Callahan and Appel, 1988), and the lack of antagonism by pirenpirone, suggest that the stimulus effects of (−) MDA resemble those of hallucinogens more closely than its enantiomer. Thus, (+) MDA probably has weaker hallucinogenic effects than (−) MDA, although the asymmetrical substitutions between both isomers of MDA and mescaline require further investigation.

Despite failing to substitute completely, cocaine engendered more drug-appropriate responding in the (+) MDA group than the (−) MDA group (Fig. 6). These results suggest that the (+), but not the (−) isomer may (or may not) resemble cocaine, depending on test conditions (Broadbent *et al.*, 1989; Emmett-Oglesby *et al.*, 1990). However, in contrast to a previous report (Glennon and Young, 1984c), both the present results (Figs 6 and 7), and those of Nichols and Oberlender (1990) indicate that the effects of

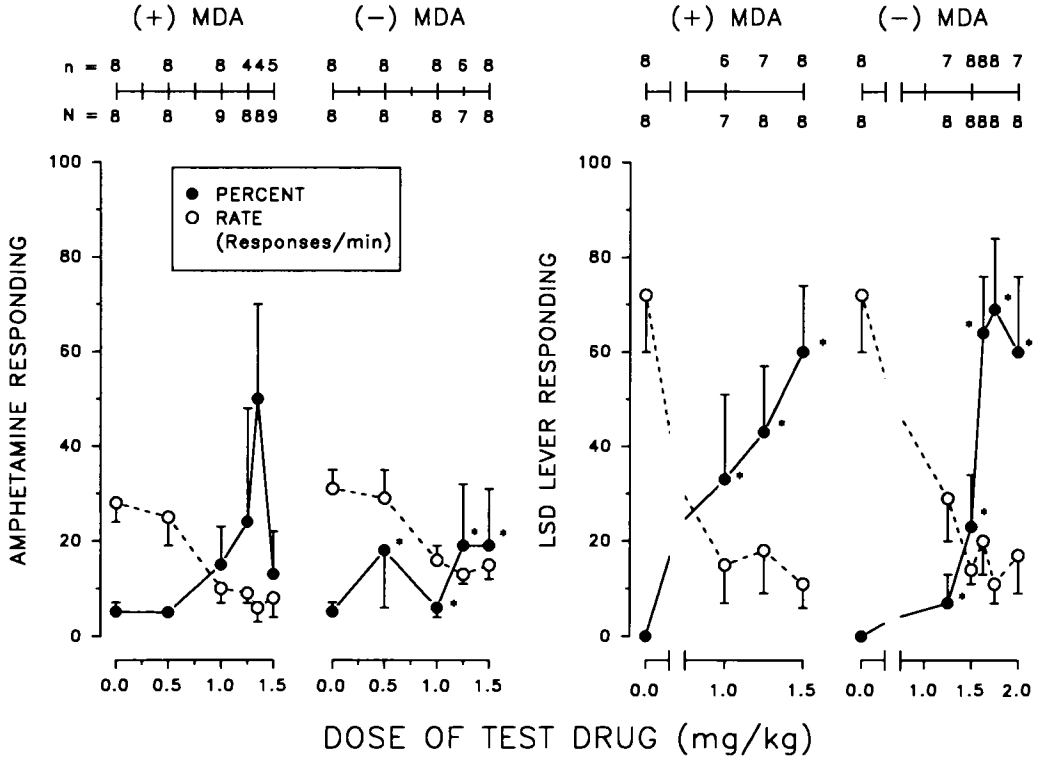

FIG. 7. Results of substitution tests with the isomers of MDA in animals trained to discriminate (+) amphetamine (1.0 mg/kg) or LSD (0.16 mg/kg) from saline. All drugs were given i.p., 15 min prior to testing. Due to the small sample size, data from (+) MDA tests in (+) amphetamine trained animals were not subjected to statistical analyses. See Fig. 2 for further details.

(+) MDA differ from those of (+) amphetamine, in that drug-appropriate responding does not exceed 60%, regardless of which drug is used to train the animals. The lack of complete substitution between these compounds cannot be attributed to saline lever biases since accurate discrimination was maintained during both training and control test sessions with (+) MDA. Moreover, while the amount of substitution of (+) MDA for (+) amphetamine (1.0 mg/kg) has been reported to depend on the administration of (+) MDA over a narrow dose range (Glennon and Young, 1984c), no more than 50% drug-appropriate responding occurred in the present study, despite the use of the same training dose of (+) amphetamine (1.0 mg/kg) and the testing of an extensive range of doses of (+) MDA. Thus, it appears that, at the doses tested, the stimulus properties of (+) MDA do not closely resemble those of (+) amphetamine.

The actions of (−) MDA generally appear to resemble those of LSD and DOM while failing to exhibit any stimulant-like properties. In contrast, the actions of (+) MDA may be unique in that the stimulus effects of (+) MDA resemble those of LSD and, under certain circumstances, those of cocaine. Further, while the effects of pirenpirone suggest that the (−) MDA cue is mediated by serotonergic (5-HT_2) mechanisms, the failure of both 5-HT and DA D_1 and DA D_2 antagonists to block the (+) MDA cue suggests that (+) MDA has more complex stimulus properties that cannot be blocked by antagonism of a single neurotransmitter.

Both (+) and (−) MDA are known to release and inhibit the uptake of 5-HT and DA (Nichols *et al.*, 1982; Johnson *et al.*, 1986; Steele *et al.*, 1987; McKenna *et al.*, 1991). While (+) MDA is the more potent isomer in inhibiting DA, it has similar effects to those of (−) MDA on 5-HT uptake (McKenna *et al.*, 1991). However, (−) MDA has a higher affinity for both 5-HT_1 and 5-HT_2 receptors than (+) MDA (Lyon *et al.*, 1986). Biochemical data such as these support the hypothesis that (−) MDA may rely more on 5-HT than (+) MDA which may interact with both 5-HT and DA neuronal systems. However, the cross-substitution of the isomers of both MDA and MDMA points to the recognition of common stimulus elements. Clearly, cocaine (or amphetamine)-like actions cannot account for this similarity. Although characterization of the stimulus effects of the isomers of MDMA may reveal LSD-like actions of these drugs, initial evidence from tests with (±) MDMA do not support this theory (Nichols *et al.*, 1986). Further, the reduced potency of (−) MDMA in the present study is not reflected in the effects of this isomer on 5-HT systems: while (−) MDMA may be less effective in releasing 5-HT (and DA) under certain circumstances than (+) MDMA and the isomers of MDA (Nichols *et al.*, 1982; McKenna *et al.*, 1991), (−) MDMA exhibits a high affinity for 5-HT receptors (Lyon *et al.*, 1986). Such findings add further weight to suggestions that these compounds may represent a pharmacologically unique class of drugs (Nichols, 1986; Nichols and Oberlender, 1990).

Some aspects of these data are interesting in that they parallel the reported subjective effects of "designer drugs" in humans. For example, distinct stimuli appear to be produced in rats by doses of both (−) and (+) MDA (1.25 mg/kg, i.p.) that are comparable on a mg/kg basis to those taken orally by recreational users (Turek *et al.*, 1974). Surprisingly, Marquardt (1978) has suggested that (−) MDA is three times more potent in humans than its enantiomer. Potency differences observed in the present study between the isomers of MDMA, however, parallel those observed in humans (Anderson *et al.*, 1978).

It is noteworthy that the majority of reports in which MDA abuse is described, involve the oral administration of racemic MDA. The results from the present study suggest that combining the hallucinogenic-like actions of (−) MDA with the weak LSD- and cocaine-like effects of (+) MDA in the racemic mixture may result in the unique subjective effects that have been described following MDA (Turek *et al.*, 1974; Weil, 1976).

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