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# A Three-Lever Operant Procedure Differentiates the Stimulus Effects of *R*(−)-MDA from *S*(+)-MDA[1]

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## ABSTRACT

1-(3,4-Methylenedioxyphenyl)-2-aminopropane (MDA) produces an effect in humans that is somewhat similar to both a hallucinogen and a central stimulant. We have previously shown that *R*(−)-MDA but not *S*(+)-MDA produces a stimulus effect in animals similar to that of the hallucinogen 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM), whereas *S*(+)-MDA but not *R*(−)-MDA produces a stimulus effect similar to that of the stimulant amphetamine. Others have suggested that the stimulus effects of the two MDA isomers may be much more similar than our results would indicate. To help clarify this issue, we have shown that rats ($n$ = 8) can be trained to discriminate 1.25 mg/kg of *R*(−)-MDA ($ED_{50}$ = 0.99 mg/kg) from 1.25 mg/kg of *S*(+)-MDA ($ED_{50}$ = 0.80 mg/kg) *versus* 0.9% saline with use of a three-lever operant procedure. To accomplish this task it was necessary to institute a separation of 4 days between injections of the isomers. In tests of stimulus substitution, the administration of various doses of DOM ($ED_{50}$ = 0.47 mg/kg), *S*(+)-DOM ($ED_{50}$ = 2.23 mg/kg) and mescaline ($ED_{50}$ = 16.04 mg/kg) produced dose-related responding on the *R*(−)-MDA-appropriate lever. In contrast, the injection of various doses of *S*(+)-amphetamine ($ED_{50}$ = 0.46 mg/kg) and cocaine ($ED_{50}$ = 6.40 mg/kg) produced dose-related responding on the *S*(+)-MDA-appropriate lever. The administration of racemic MDA, at twice the isomer training dose, resulted in the animals dividing their responses closely between the *R*(−)-MDA- and *S*(+)-MDA-designated levers. In antagonism tests, the serotonin-2 antagonist pirenperone blocked the stimulus effect of 1.25 mg/kg of *R*(−)-MDA but had no effect on the stimulus effect of 1.25 mg/kg of *S*(+)-MDA. Taken together, these data support the contention that each optical isomer of MDA can produce a markedly different stimulus effect.

Phenylalkylamines, depending on what substituents are present in the molecule, are capable of producing a wide variety of pharmacological effects. Prominent among these are their central stimulant and hallucinogenic effects. For example, AMPH is a prototypical phenylalkylamine stimulant, whereas its chemically related derivative DOM is a prototypical phenylalkylamine hallucinogen. As closely structurally related as they are, there is no compelling evidence that AMPH and DOM produce similar central effects in humans. Likewise, the stimulus properties of these two agents also differ in rats. For example, *S*(+)-AMPH-stimulus substitution fails to occur with DOM, and DOM-stimulus substitution fails to occur with *S*(+)-AMPH (Glennon and Young, 1984a; Silverman and Ho, 1980). Moreover, the structure-activity relationships for producing an *S*(+)-AMPH-like or DOM-like stimulus effect are also different, as are their proposed stimulus mechanisms of action (Glennon *et al.*, 1983a; Young and Glennon, 1986). Dopamine antagonists attenuate the *S*(+)-AMPH stimulus, whereas they have little to no effect on the DOM stimulus (*e.g.*, Glennon, 1989; Young and Glennon, 1986). In contrast, 5-$HT_{2A}$ (or mixed 5-$HT_{2A}$/5-$HT_{2C}$) antagonists block the DOM stimulus but have no effect on the *S*(+)-AMPH stimulus (*e.g.*, Glennon and Hauck, 1985; Glennon *et al.*, 1983a; Glennon *et al.*, 1983b; Young *et al.*, 1980). Thus, *S*(+)-AMPH and DOM represent prototypical centrally active phenylalkylamines capable of producing distinct pharmacological effects.

MDA is the methylenedioxy analog of AMPH; MDA also bears some structural similarity to DOM (fig. 1). Thus, it is probably not altogether surprising that MDA produces a stimulus effect similar to each of the two prototypical phenylalkylamines. That is, both the *S*(+)-AMPH stimulus and the DOM stimulus substitute to racemic MDA in a dose-related fashion (Glennon *et al.*, 1982; Glennon and Young, 1984a). Interestingly, the *S*(+)-AMPH stimulus and the DOM stimulus each substitute only to one of the optical isomers of MDA; the "active" isomer depends on the particular stimulus. The *S*(+)-AMPH-like stimulus effect of MDA appears to reside primarily with its *S*(+)-isomer, whereas the *R*(−)-isomer seems primarily responsible for the DOM-like stimulus effect

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**ABBREVIATIONS:** DOM, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane; 5-HT, serotonin; MDA, 1-(3,4-methylenedioxyphenyl)-2-aminopropane; AMPH, amphetamine; LSD, (+)-lysergic acid diethylamide.

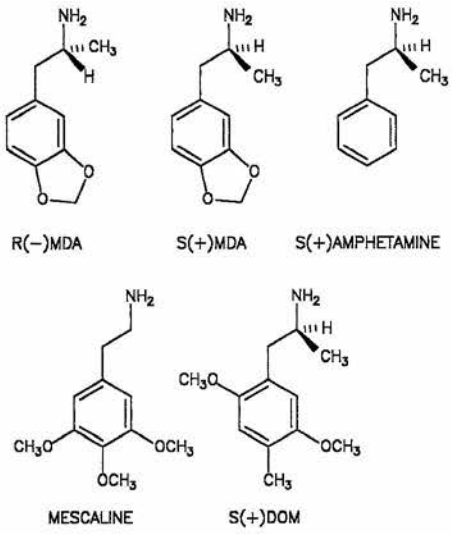


**Fig. 1.** Chemical structures of some of the agents used in this study.

(Glennon *et al.*, 1982; Glennon and Young, 1984c). Indeed, for the >200 phenylalkylamines we have examined to date, MDA is the only example that produces both types of stimulus effects.

Animals also have been trained to discriminate MDA from vehicle. Consistent with the findings above the MDA stimulus substituted both to *S*(+)-AMPH and DOM; furthermore, it also substituted to other central stimulants and hallucinogens, such as cocaine and LSD, respectively (Glennon and Young, 1984a, b). These results confirm the unique pharmacology of MDA and further suggest that it produces dual stimulus effects consisting of a stimulant (*i.e.*, AMPH-like) component and a hallucinogenic (*i.e.*, DOM-like) component. Several other investigators have since confirmed many of these observations. For example, Kamien *et al.* (1986) demonstrated that MDA produces an *S*(+)-AMPH-like stimulus effect in monkeys trained to discriminate *S*(+)-AMPH from vehicle, and Evans and Johanson (1986) reported similar results with pigeons. Moreover, in rats trained to discriminate cocaine from vehicle, stimulus substitution occurs with *S*(+)-MDA (Broadbent *et al.*, 1989), whereas in LSD-trained rats, stimulus substitution occurs to racemic MDA, *R*(−)-MDA, but not to *S*(+)-MDA (Callahan and Appel, 1988; Oberlender and Nichols, 1988). In contrast, there are some reports that appear inconsistent with these observations. For example, there is one report that both optical isomers produce complete mescaline-like appropriate responding in mescaline-trained rats (Callahan and Appel, 1988). More recently, Broadbent *et al.* (1992) trained rats to discriminate either *R*(−)-MDA, *S*(+)-MDA, LSD or *S*(+)-AMPH from vehicle. They concluded that in *R*(−)-MDA-trained animals, stimulus substitution occurred with *S*(+)-MDA, DOM and LSD, but not with mescaline, *S*(+)-AMPH and cocaine. In comparison, *S*(+)-MDA-trained rats reportedly recognized *R*(−)-MDA and LSD, but not DOM, mescaline, *S*(+)-AMPH and cocaine. Furthermore, they reported that neither MDA isomer substituted completely in either LSD- or *S*(+)-AMPH-trained rats. Taken together, these latter findings would argue that *S*(+)- and *R*(−)-MDA produce similar stimulus effects, or at least that their stimulus properties share greater similarity than difference. Possible explanations for these apparent discrepancies might be found in variations in training drug dose, test drug dose and schedules of reinforcement; experimental factors that are thought to control generalization results (*e.g.*, Terry *et al.*, 1994; White and Appel, 1982). Nevertheless, this issue remains unresolved and requires further study.

In general, animals cannot be trained to discriminate between two agents that produce similar stimulus effects. Thus, to determine whether or not the stimulus effect of *R*(−)-MDA is similar to the stimulus effect of *S*(+)-MDA, we attempted to train a group of rats to discriminate *R*(−)-MDA from *S*(+)-MDA from saline in a three-lever drug discrimination paradigm. If animals could be trained successfully, tests of stimulus substitution with a central stimulant, such as *S*(+)-AMPH or cocaine, and a hallucinogenic agent, such as DOM or mescaline, would further aid in defining the stimulus properties of the two MDA optical isomers.

## Materials and Methods

**Subjects.** Eight experimentally naive, male albino Sprague-Dawley rats (Charles River Labs.) weighing 350 to 375 g at the beginning of the experiment were used. Rats were housed individually and had free access to water, but were gradually food deprived to approximately 80% of their free-feeding weights before training was begun. The colony in which the animals were housed was kept at a constant temperature (21–23°C) and humidity (40–50%); lights were on from 6:00 A.M. to 6:00 P.M..

**Apparatus.** Behavioral testing was conducted in three operant chambers (BRS/LVE, Model RTC-025), housed within light- and sound-attenuating outer chambers. One wall of each operant chamber was fitted with two levers (BRS/LVE, Model CRL-005) and a dipper device (BRS/LVE, Model SLD-002), centered equidistant between the levers for delivery of reinforcement (0.01 ml of sweetened milk). A third lever (BRS/LVE, Model CRL-005) was mounted 5 cm above the top of each dipper aperture. Illumination of each chamber was provided by an overhead 28-V houselight. Solid-state and electromechanical programming and recording equipment were used and these were housed in the same room as the operant chambers.

**Initial training.** The illumination of the house light signaled the beginning of an experimental session. The rats were trained to respond on each lever for sweetened milk under a fixed ratio 1 (FR1) schedule of reinforcement. The FR1 requirement was increased gradually until all rats were responding reliably under a variable interval 15 sec (VI 15 s) schedule of reinforcement. After lever responding was established, each daily session was preceded by an i.p. injection of either *R*(−)-MDA (1.25 mg/kg), *S*(+)-MDA (1.25 mg/kg) or saline (0.9% NaCl) with only the stimulus-appropriate lever present (*i.e.*, left, center or right lever). A presession injection interval of 15 min was used; during this interval the animals were in their home cages. Training sessions were of 15 min duration, 5 days per week. Animals received four consecutive *R*(−)-MDA sessions, a single saline session, followed by four consecutive *S*(+)-MDA sessions. To establish reliable responding, each treatment condition [*S*(+)-MDA, *R*(−)-MDA and saline] was presented in random order for four additional sessions. For three of the rats, responding on the right-side, center and left-side lever was reinforced after the administration of *R*(−)-MDA, *S*(+)-MDA and saline, respectively. For three other rats, responding on the right-side, center and left-side lever was reinforced after the administration of saline, *R*(−)-MDA and *S*(+)-MDA respectively. For the remaining two rats, responding on the right-side, center and left-side lever was reinforced after the administration of *S*(+)-MDA, saline and *R*(−)-MDA, respectively.

**Discrimination learning.** The three levers were then presented simultaneously. Rats were required to respond on the stimulus-appropriate (correct) lever for reinforcement after each of the three

conditions. Incorrect responses had no programmed consequences. At least once per 2 weeks, discrimination learning was assessed under each condition during an initial 2.5-min nonreinforced (*i.e.*, extinction) session followed by a 12.5-min training session. Data collected during the extinction session included total responses (expressed as mean responses per minute) and percent drug-lever responding (*i.e.*, number of responses on the treatment-designated lever as a percent of the total number of responses). Initially, the two MDA isomers and saline were administered in a random order with the restriction that no treatment be presented for more than three consecutive sessions. During 5 months of training, animals did not learn to make their responses consistently on the appropriate lever after administration of the MDA isomers and saline. It was then decided that a time lapse of several days might be beneficial in establishing the discrimination. The final separation time for injections of MDA's isomers was 4 days; saline training sessions were conducted on intervening days. After 5 to 6 months of training sessions, rats consistently made ≥80% of their total responses on the drug-appropriate lever after administration of *R*(−)-MDA (84 ± 3), *S*(+)-MDA (90 ± 4) or saline (94 ± 2) and ≤20% of their responses on the incorrect levers.

**Stimulus substitution and antagonism studies.** Maintenance of the *R*(−)-MDA/*S*(+)-MDA/saline discrimination was ensured by continuation of training sessions throughout this portion of the study. The separation of 4 days between injections of the isomers remained in effect. Training sessions were conducted on the 9 to 12 days before a stimulus substitution or antagonism test session. On at least two of these days, half the animals received 1.25 mg/kg *S*(+)-MDA and the other half received 1.25 mg/kg *R*(−)-MDA (the animals' injections were counterbalanced); after a 2.5-min extinction period, training was continued for an additional 12.5 min as described above. Saline training sessions were conducted on intervening days; at least two tests with the saline training stimulus were conducted before a substitution test. Animals not meeting the above criteria (*i.e.*, ≥80%) under all injection treatments were not used in that week's stimulus substitution or antagonism test. Stimulus substitution test sessions were conducted every 10th to 13th day by administration of a dose of *R*(−)-MDA (0.25, 0.75, 1.0, 1.25 and 1.40 mg/kg), *S*(+)-MDA (0.25, 0.75, 1.0 and 1.25 mg/kg) or a challenge drug; animals were allowed 2.5 min to respond under nonreinforced conditions and were then returned to their individual home cages. Test compounds included: DOM (0.25, 0.50 and 1.0 mg/kg), *S*(+)-DOM (1.0, 2.0, 3.0 and 4.0 mg/kg), *S*(+)-AMPH (0.25, 0.50, 0.75 and 1.0 mg/kg), cocaine (2.0, 6.0, 8.0 10.0 and 13.0 mg/kg), (±)-MDA (0.25, 0.75, 1.25, 2.0 and 2.5 mg/kg) and mescaline (10.0, 15.0, 20.0 and 22.5 mg/kg). Doses of these agents were administered in a random sequence 15 min before testing. Stimulus substitution was said to have occurred when the animals made ≥80% of their responses on a drug-appropriate lever. Where stimulus substitution or antagonism (see below) occurred, effective dose 50 ($ED_{50}$) or antagonism dose 50 ($AD_{50}$) values, respectively, were calculated by the method of Finney (1964) and reflect the dose at which the animals would be expected to make 50% of their responses on a drug-appropriate lever. Stimulus antagonism studies were conducted as described for the stimulus substitution studies except that doses of the 5-$HT_2$ antagonist pirenperone were administered 45 min before an injection of 1.25 mg/kg of *S*(+)-MDA, 1.25 mg/kg of *R*(−)-MDA or saline; 15 min later, animals were placed in an operant chamber for a 2.5-min extinction session.

**Drugs.** DOM HCl, *S*(+)-DOM HCl, (±)-MDA HCl, *S*(+)-MDA HCl and *R*(−)-MDA HCl were obtained from the National Institute on Drug Abuse. Pirenperone tartrate was a gift from Janssen Pharmaceutica (Beerse, Belgium). *S*(+)-AMPH sulfate, cocaine HCl and mescaline HCl were purchased from Sigma Chemical Co. (St. Louis, MO). All solutions were prepared fresh daily in sterile saline and administered *via* the i.p. route in a 1 ml/kg injection volume. Doses of each compound are based on the weight of the salt.

## Results

**Discrimination training.** The investigation began with the isomers of MDA and saline being administered in a random order with the restriction that no treatment be presented for more than three consecutive sessions. That procedure proved unsuccessful in establishing the three-lever discrimination (data not presented). A separation time, of 2 days and then 3 days, was then initiated between the administration of the isomers. This, too, did not result in the rats learning the discrimination (data not shown). The animals did learn the task, however, with a 4-day separation between injections of the isomers. Thus, the animals were trained to discriminate 1.25 mg/kg of *S*(+)-MDA from *R*(−)-MDA and saline such that they made ≥80% of their responses on the *S*(+)-MDA-designated lever and <20% of their responses on the other two levers when administered the training dose of *S*(+)-MDA (fig. 2). Similarly, rats were able to differentiate 1.25 mg/kg of *R*(−)-MDA from *S*(+)-MDA and saline in that they made ~80% of their responses on the *R*(−)-MDA-designated lever and ~20% of their responses on the other two levers when administered the training dose of *R*(−)-MDA (fig. 2). Finally, rats were able to discriminate 1.0 ml/kg of saline from *S*(+)-MDA and *R*(−)-MDA such that they made >95% of their responses on the saline-designated lever and <5% of their responses on the other two levers when administered saline (fig. 2). Response rates (mean responses per minute) were not substantially different under drug and saline conditions: *S*(+)-MDA = 12 ± 3, *R*(−)-MDA = 13 ± 3, saline = 14 ± 2.

**Stimulus substitution tests.** Administration of *S*(+)-MDA ($ED_{50}$ = 0.80 mg/kg) or *R*(−)-MDA ($ED_{50}$ = 0.99 mg/kg) doses lower than that of the training dose produced decreased percent responding on the drug-appropriate lever (figs. 3 and 4). The dose-response study with *R*(−)-MDA also included the administration of a dose of *R*(−)-MDA higher than that of the training dose: at a dose of 1.4 mg/kg, *R*(−)-MDA produced 84% *R*(−)-MDA-designated lever responding. Tests of stimulus substitution were conducted with *S*(+)-AMPH, cocaine, DOM, *S*(+)-DOM, mescaline and (±)-MDA (figs. 5–10). Figures 5 and 6 show that *S*(+)-MDA-stimulus substitution occurs to *S*(+)-AMPH ($ED_{50}$ = 0.46 mg/kg) and cocaine ($ED_{50}$ = 6.40 mg/kg). Response rates were not substantially different under *S*(+)-AMPH, *S*(+)-MDA and saline treatments; a depressed response rate (~50%) was produced, however, by the cocaine dose (13.0 mg/kg) that produced >80% *S*(+)-MDA-like responding. Both figures also show that *S*(+)-AMPH and cocaine, under all doses tested, produced no more than 18% *R*(−)-MDA-appropriate lever responding. Figures 7 to 9 reveal that *R*(−)-MDA-like responding occurred after administration of DOM ($ED_{50}$ = 0.47 mg/kg), *S*(+)-DOM ($ED_{50}$ = 2.23 mg/kg) and mescaline ($ED_{50}$ = 16.04 mg/kg). Response rates were not appreciably altered under DOM, *R*(−)-MDA and saline conditions; decreased response rates (~40–60%) were observed, however, with doses of *S*(+)-DOM (3.0 and 4.0 mg/kg) and mescaline (20.0 and 22.5 mg/kg) that produced substantial (>60%) *R*(−)-MDA-like responding. Maximal *S*(+)-MDA-designated lever responding of approximately 40% was observed at doses of 0.5 mg/kg of DOM, 2.0 mg/kg of *S*(+)-DOM and 15.0 of mg/kg mescaline; one rat responded 82% on the *S*(+)-MDA-designated lever after the administration of 15 mg/kg of mesca-

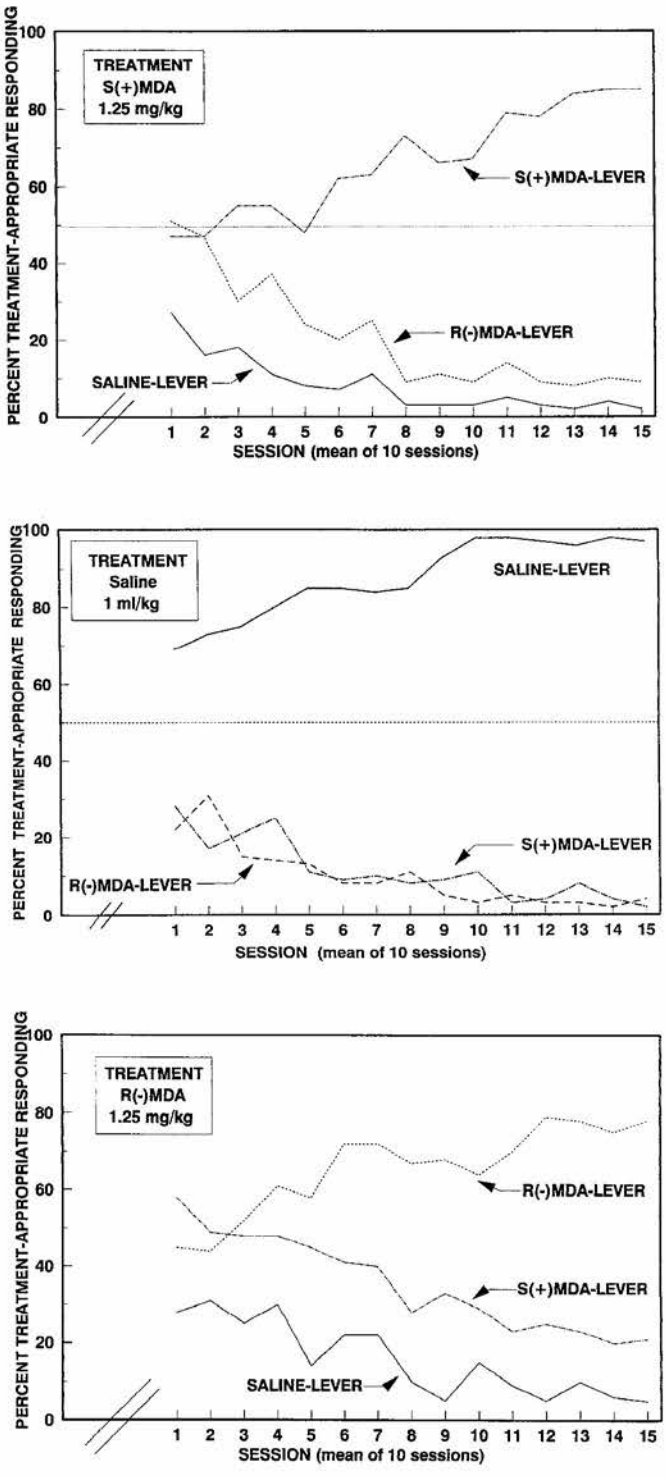


**Fig. 2.** Learning curves for the acquisition of the *S*(+)-MDA *vs.* *R*(−)-MDA *vs.* saline three-lever discrimination. Ordinate: Mean ($n$ = 8) percentage of responses made on each of the treatment-designated levers after the administration of 1.25 mg/kg of *S*(+)-MDA (top), 1.25 mg/kg of *R*(−)-MDA (bottom) and 1 ml/kg of saline (middle). Data were collected during 2.5-min extinction periods initiated at the beginning of a training session. Abscissa: each number represents a successive block of 10 sessions for a total of 150 sessions.

line. Higher doses of these agents produced decreased percent *S*(+)-MDA-appropriate lever responding in all rats. The last stimulus substitution test studied the effects of racemic MDA doses up to 2.5 mg/kg, *i.e.*, twice the training dose of

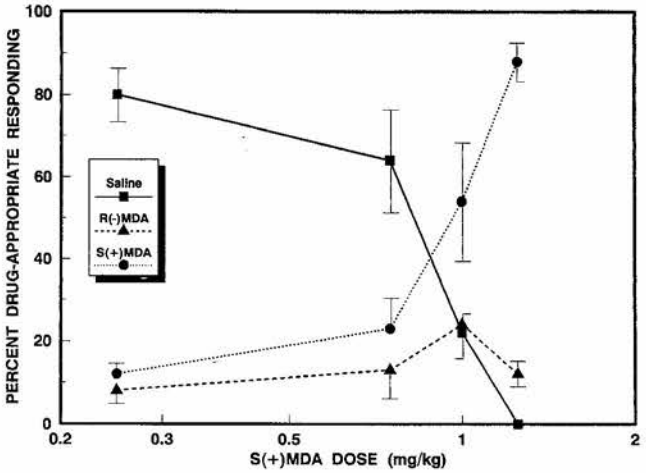


**Fig. 3.** Results of dose-response tests with *S*(+)-MDA in rats ($n$ = 8 at each dose) trained to discriminate *S*(+)-MDA (1.25 mg/kg) from *R*(−)-MDA (1.25 mg/kg) from saline. Ordinate: mean (±S.E.M.) percent drug-appropriate responding on each of the three levers. Solid line with solid squares reflects percent saline-appropriate responding, dashed line with solid triangles reflects percent *R*(−)-MDA-appropriate responding, dotted line with solid circles reflects percent *S*(+)-MDA-appropriate responding, during test (2.5-min extinction) sessions. Abscissa: dose in milligrams per kilogram of body weight plotted on a $\log_{10}$ scale.

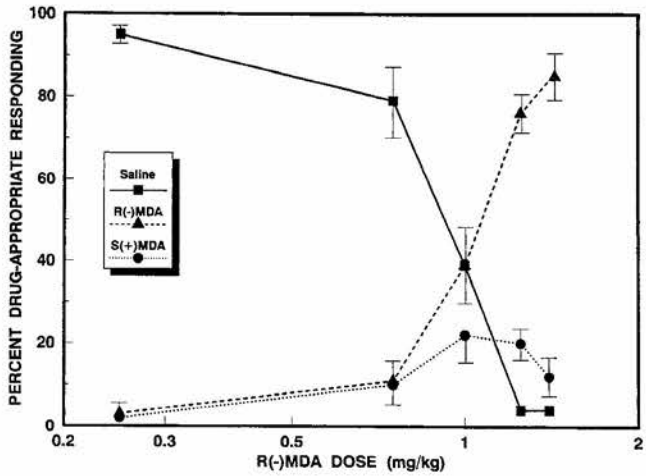


**Fig. 4.** Results of dose-response tests with *R*(−)-MDA in rats ($n$ = 8 at each dose) trained to discriminate *S*(+)-MDA (1.25 mg/kg) from *R*(−)-MDA (1.25 mg/kg) from saline. See figure 3 for other details.

each MDA isomer. The results in figure 10 show that 0.25 to 1.25 mg/kg of (±)-MDA produced predominantly saline-appropriate responding. At higher doses, percent drug-lever responding shifted to the levers designated for the MDA isomers: doses of 2.0 and 2.5 mg/kg of (±)-MDA produced 35% and 44% *R*(−)-MDA-like responding, respectively, and 41% and 54% *S*(+)-MDA-like responding, respectively. Response rates were not substantially different under drug and saline conditions. A summary of $ED_{50}$ values or maximal percent isomer-appropriate responding is presented in table 1.

**Stimulus antagonism tests.** Tests of stimulus antagonism were conducted by administration of doses of pirenperone in combination with the training dose of *R*(−)-MDA, *S*(+)-MDA and 1 ml/kg of saline. Figure 11 shows that pirenperone is an antagonist ($AD_{50}$ = 15 μg/kg) of the *R*(−)-MDA stimulus; as percent *R*(−)-MDA-appropriate responding de-

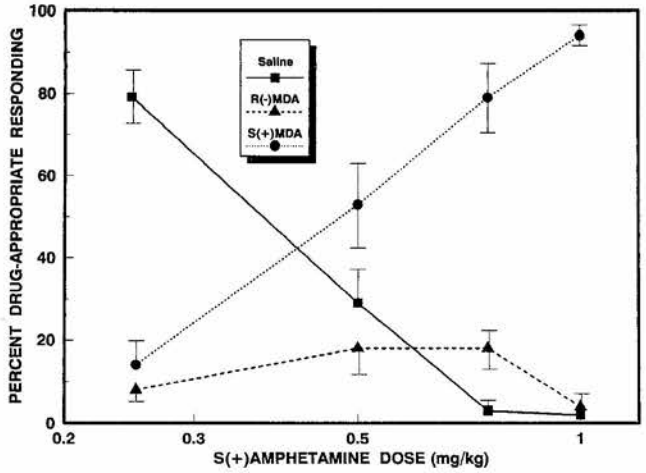


**Fig. 5.** Results of stimulus substitution tests with *S*(+)-AMPH in rats ($n$ = 5 or 6 at each dose) trained to discriminate *S*(+)-MDA (1.25 mg/kg) from *R*(−)-MDA (1.25 mg/kg) from saline. See figure 3 for other details.

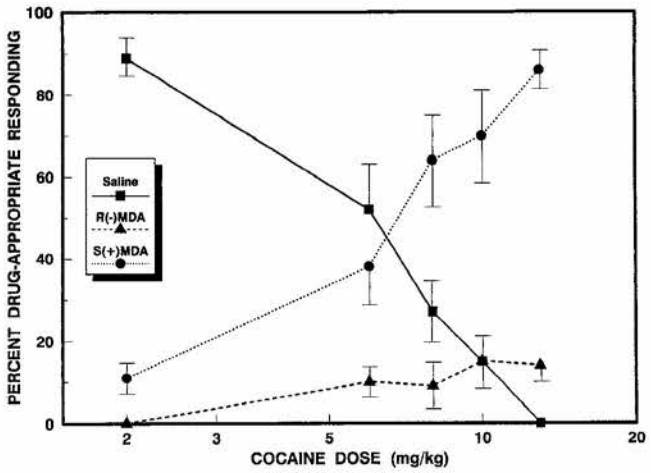


**Fig. 6.** Results of stimulus substitution tests with cocaine in rats ($n$ = 5 at each dose) trained to discriminate *S*(+)-MDA (1.25 mg/kg) from *R*(−)-MDA (1.25 mg/kg) from saline. See figure 3 for other details.

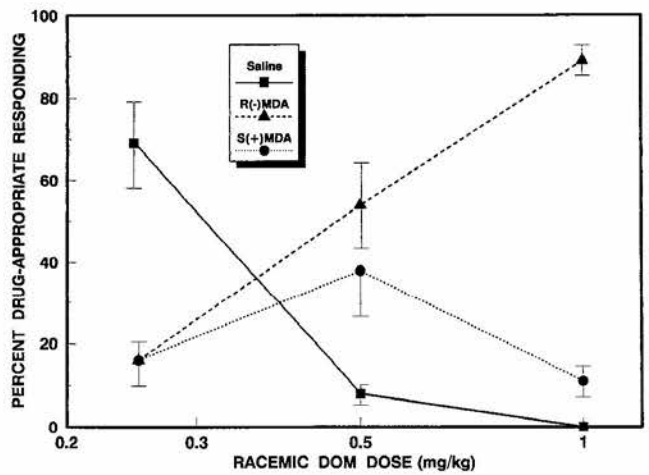


**Fig. 7.** Results of stimulus substitution tests with racemic DOM in rats ($n$ = 6 at each dose) trained to discriminate *S*(+)-MDA (1.25 mg/kg) from *R*(−)-MDA (1.25 mg/kg) from saline. See figure 3 for other details.

creased, percent saline-appropriate responding increased. *S*(+)-MDA-appropriate responding was below 20%. The rats response rates were not appreciably altered after each dose of

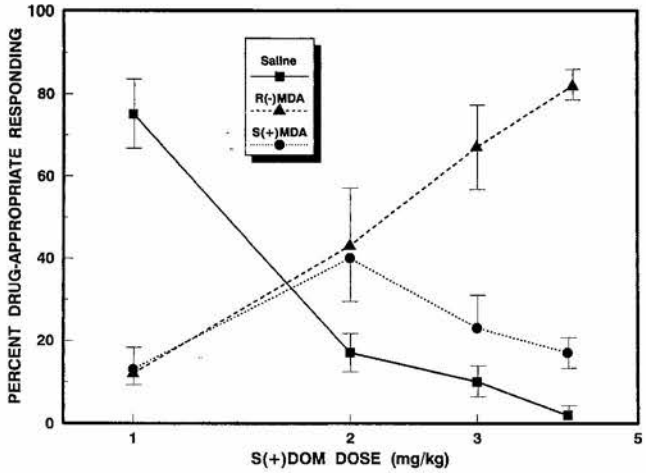


**Fig. 8.** Results of stimulus substitution tests with *S*(+)-DOM in rats ($n$ = 5 at each dose) trained to discriminate *S*(+)-MDA (1.25 mg/kg) from *R*(−)-MDA (1.25 mg/kg) from saline. See figure 3 for other details.

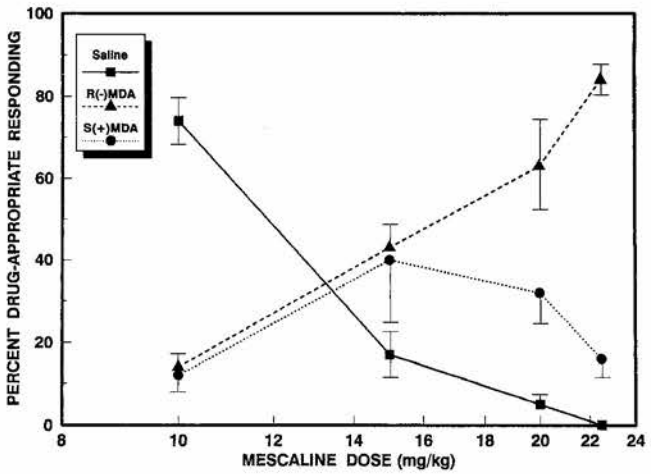


**Fig. 9.** Results of stimulus substitution test with mescaline in rats ($n$ = 3 at each dose) trained to discriminate *S*(+)-MDA (1.25 mg/kg) from *R*(−)-MDA (1.25 mg/kg) from saline. See figure 3 for other details.

pirenperone and subsequent injections of 1.25 mg/kg of *R*(−)-MDA. Figure 12 shows that administration of 5 to 200 μg/kg of pirenperone in combination with 1.25 mg/kg of *S*(+)-MDA had no effect on *S*(+)-MDA-appropriate responding: responding was 88% to 94% on the *S*(+)-MDA-appropriate lever. Responding on the *R*(−)-MDA-designated lever or the saline-appropriate lever was below 20% at each pirenperone dose in combination with 1.25 mg/kg of *S*(+)-MDA. Although high percentages of *S*(+)-MDA-appropriate responding were generated after the administration of 100 or 200 μg/kg of pirenperone in combination with 1.5 mg/kg of *S*(+)-MDA, the animals' response rates were greatly decreased; because response rates were severely depressed at the combination of 200 μg/kg of pirenperone and 1.25 mg/kg of *S*(+)-MDA, additional doses of pirenperone were not examined. Lastly, administration of various doses of pirenperone (10–200 μg/kg) in combination with saline resulted in saline-like responding. Response rates were depressed (~30–60%), however, following injections of 100 or 200 μg/kg of pirenperone and saline (percent responding and response rate data not presented).

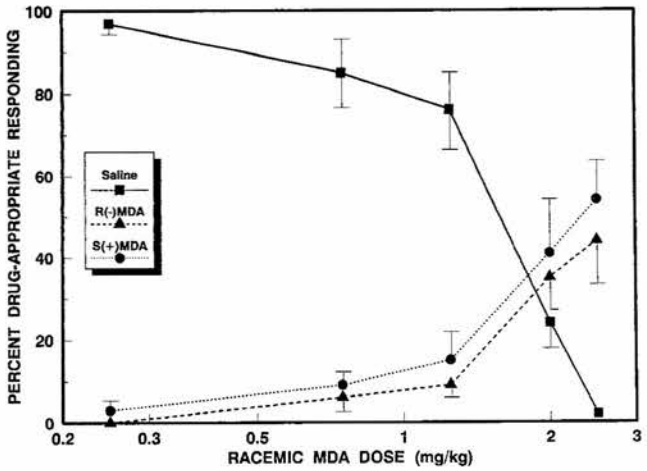


**Fig. 10.** Results of dose-response tests with (±)-MDA in rats ($n = 3$ at each dose) trained to discriminate S(+)-MDA (1.25 mg/kg) from R(−)-MDA (1.25 mg/kg) from saline. See figure 3 for other details.

TABLE 1

**A summary of $ED_{50}$ values or maximal percent isomer-appropriate responding in rats trained to discriminate 1.25 mg/kg of R(−)-MDA from 1.25 mg/kg of S(+)-MDA from saline[a]**

| Test Drug | Maximal % R(−)-MDA-appropriate Responding, or $ED_{50}$ Value | Maximal % S(+)-MDA-appropriate Responding, or $ED_{50}$ Value |
|---|---|---|
| MDA isomers | | |
| S(+)-MDA | 24% | 0.80 mg/kg |
| R(−)-MDA | 0.99 mg/kg | 22% |
| Stimulants | | |
| S(+)-AMPH | 18% | 0.46 mg/kg |
| Cocaine | 15% | 6.40 mg/kg |
| Hallucinogens | | |
| DOM | 0.47 mg/kg | 38% |
| S(+)-DOM | 2.23 mg/kg | 40% |
| Mescaline | 16.04 mg/kg | 40% |

[a] Where stimulus generalization occurred, an $ED_{50}$ value in milligrams per kilogram is provided. Where generalization failed to occur, maximal percent isomer-appropriate responding is shown.

## Discussion

Although racemic, S(+)- and R(−)-MDA have been used as training drugs in previous drug discrimination studies, this is the first time that a group of animals has been trained to discriminate R(−)-MDA from S(+)-MDA from saline by use of a three-lever drug discrimination task. Indeed, to our knowledge, this is the first demonstration that optical isomers of the same compound can be differentiated with a three-lever procedure. To establish the discrimination, however, it was necessary to institute a time lapse of 4 days between injections of the isomers; rats did not learn the task with shorter (*i.e.*, 1–3 days) injection intervals. Unfortunately, we are unable to provide an explanation for the need of such a treatment factor. Perhaps a future investigation(s) of the isomers' distribution and/or metabolism under conditions similar to the present study may provide a clue(s). The occurrence of stimulus substitution suggests that a challenge drug is capable of producing a stimulus effect similar to that produced by a particular training drug. Based on previous drug discrimination studies we, and others, have contended that S(+)-MDA produces primarily, but not necessarily exclusively, a stimulant (*i.e.*, AMPH-like) stimulus effect and that R(−)-MDA produces primarily, but not necessarily exclusively, a hallucinogenic (*i.e.*, DOM-like) stimulus effect (Glennon *et al.*, 1982; Glennon and Young, 1984c; Callahan and Appel, 1988; Oberlender and Nichols, 1988; Broadbent *et al.*, 1989). Other investigations have concluded that S(+)- and R(−)-MDA share greater similarity than difference in their discriminative stimulus effects (Callahan and Appel, 1988; Broadbent *et al.*, 1992). Especially relevant is the recent study by Broadbent *et al.* (1992) that used two groups of rats trained to discriminate 1.25 mg/kg of either R(−)-MDA or S(+)-MDA from saline. In tests of stimulus substitution, complete (>80%) R(−)-MDA-like responding occurred with S(+)-MDA and DOM whereas considerable substitution (*i.e.*, ~70% R(−)-MDA-appropriate responding) occurred with mescaline. In comparison, no more than 40% R(−)-MDA-like responding occurred with S(+)-AMPH and cocaine. In the present three-lever study, complete (>80%) R(−)-MDA-like responding occurred with DOM and mescaline (figs. 7 and 9 and table 1), whereas no more than 20% R(−)-MDA-appropriate responding was generated by S(+)-AMPH and cocaine (figs. 5 and 6, and table 1). In Broadbent's second group of rats trained to discriminate S(+)-MDA from saline, full S(+)-MDA-like responding occurred with R(−)-MDA whereas substantial substitution (*i.e.*, 75% S(+)-MDA-appropriate responding) occurred with cocaine. Partial substitution was observed with DOM and S(+)-AMPH (*i.e.*, 65% and 60% S(+)-MDA-like responding, respectively), whereas no more than 40% S(+)-MDA-like responding occurred with mescaline. In the present study, complete S(+)-MDA-like responding occurred with cocaine and S(+)-AMPH (figs. 5 and 6 and table 1) whereas approximately 40% S(+)-MDA-like responding was generated both by DOM and mescaline (figs. 7 and 9 and table 1). Thus, an overall comparison of the actual test drug results by Broadbent *et al.* (1992) and the present results (including antagonism tests in both studies; see below) reveals fairly good agreement between the two studies. The notable exception is the Broadbent *et al.* (1992) finding that cross-substitution occurs between R(−)-MDA and S(+)-MDA regardless of which is used as the training drug. In their study, test sessions with R(−)-MDA in S(+)-MDA-trained rats and S(+)-MDA in R(−)-MDA-trained rats were conducted once or twice per week in addition to training sessions with the particular MDA isomer that was being used as training drug. In light of the present finding that a time lapse of 4 days was needed to establish the discrimination between the isomers, perhaps the shorter intervals between isomer injections in the Broadbent study may have affected their cross-substitution results. Obviously, further research will be needed to evaluate that explanation.

On the other hand, it seems difficult to reconcile Callahan and Appel's (1988) finding that mescaline-trained rats completely recognized S(+)-MDA with the present finding, and the Broadbent *et al.* (1992) result that significant S(+)-MDA-like responding does not occur with mescaline. However, an examination of training drug dose and test drug dose may be helpful in resolving these differences. In the Callahan and Appel study, rats were trained to discriminate 10 mg/kg of mescaline from saline, and complete stimulus substitution was generated both by S(+)-MDA and R(−)-MDA. In the present three-lever task, 10 mg/kg of mescaline produced approximately 15 to 20% responding on each of the drug [*i.e.*, R(−)-MDA and S(+)-MDA] appropriate levers. Likewise, in

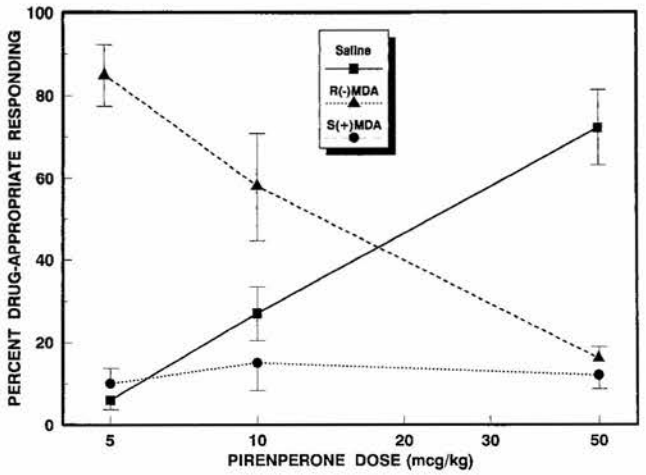


**Fig. 11.** Results of antagonism tests with microgram (mcg/kg) doses of pipenperone given before the administration of 1.25 mg/kg of *R*(−)-MDA in rats ($n = 5$ at each dose) trained to discriminate *S*(+)-MDA (1.25 mg/kg) from *R*(−)-MDA (1.25 mg/kg) from saline. See figure 3 for other details.

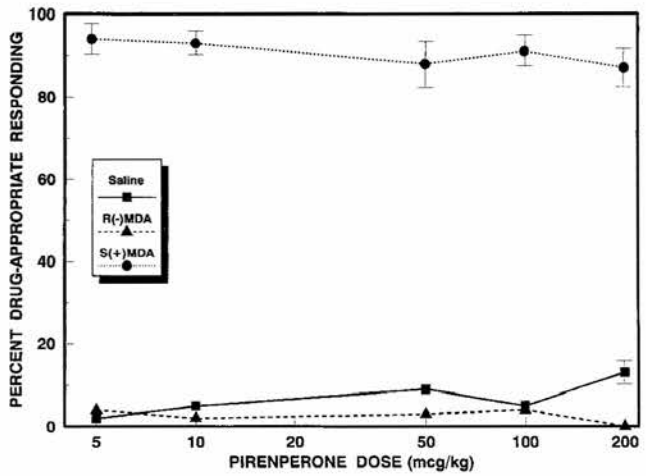


**Fig. 12.** Results of antagonism tests with microgram (mcg/kg) doses of pirenperone given before the administration of 1.25 mg/kg of *S*(+)-MDA in rats ($n = 5$ at each dose) trained to discriminate *S*(+)-MDA (1.25 mg/kg) from *R*(−)-MDA (1.25 mg/kg) from saline. See figure 3 for other details.

the Broadbent *et al.* (1992) study, 10 mg/kg of mescaline occasioned approximately 40% to 50% drug appropriate responding in each group of rats trained to discriminate either *R*(−)-MDA or *S*(+)-MDA from saline. Thus, in the latter two studies animals were unable to differentiate 10 mg/kg of mescaline as being primarily either *S*(+)-MDA-like or *R*(−)-MDA-like; higher doses of mescaline were needed to establish it as being *R*(−)-MDA-like. Consequently, a future investigation(s) that might use a three-lever task in rats, or separate groups of rats, trained to discriminate a "low" (*e.g.*, 10 mg/kg) and a "high" (*e.g.*, >20 mg/kg) dose of mescaline from saline might be able to determine more precisely if mescaline's training dose is a critical factor in determining substitution results with MDA's optical isomers.

An early discrimination study by Tilson and co-workers (1975) suggested that DOM might possess an AMPH-like discriminative stimulus effect. Although other investigators have failed to obtain supporting evidence for this [*e.g.*, see review by Glennon *et al.* (1983a) for further discussion], the design of the present study is uniquely constructed to readdress this issue. Because *S*(+)-DOM is four times less potent than racemic DOM in drug discrimination studies with rats trained to discriminate DOM from saline (Glennon *et al.*, 1983a), and because an optimal AMPH-like stimulus effect is associated with the *S*(+)-isomer of AMPH-like agents, we examined the stimulus activity of *S*(+)-DOM in the MDA-isomer trained animals, with the expectation that the DOM-like stimulus effect should be minimized, and any AMPH-like stimulus effect should be maximized, in *S*(+)-DOM. As shown in figure 8 and table 1, *S*(+)-DOM results in *R*(−)-MDA-stimulus substitution, and fails to produce greater *S*(+)-MDA-appropriate responding than racemic DOM. Interestingly, the greater potency of DOM relative to *S*(+)-DOM in the present study (4.7-fold) is similar to that found in DOM-trained rats (Glennon *et al.*, 1983a).

When (±)-MDA has been used as the training agent in drug discrimination studies, stimulus substitution occurs with amphetamine, cocaine, LSD and DOM (Glennon and Young, 1984a, b). Likewise, in humans, (±)-MDA produces effects that are similar both to hallucinogenic agents, such as LSD, and central stimulants, such as amphetamine (Shulgin, 1978). Therefore, if the isomers of MDA are exerting markedly different stimulus effects in the present three-lever discrimination task, then the administration of (±)-MDA to the rats, at twice the training dose of each MDA isomer, should result in percent responding that does not substantially favor either isomer-designated lever. Such was found to be the case. Specifically, 2.5 mg/kg of (±)-MDA produced 44% and 54% *R*(−)-MDA and *S*(+)-MDA lever appropriate responding, respectively (fig. 10 and table 1). Thus, these data further indicate the differences in the stimulus effects of MDA's isomers and, to a lesser extent, the dual stimulus nature of racemic MDA.

Finally, evidence that the stimulus effect produced by certain hallucinogenic agents is mediated by serotonergic mechanisms is derived from the finding that these stimuli can be blocked by the administration of serotonin 5-$HT_{2A}$/5-$HT_{2C}$ (formerly 5-$HT_{1C}$; see Hartig, 1989) receptor antagonists, such as pirenerone and ketanserin. The stimulus effects of DOM and LSD have been blocked in such a manner (Colpaert *et al.*, 1982; Glennon *et al.*, 1983b). To further differentiate the stimulus effects of MDA's isomers in the present three-lever task, pirenperone was administered before the rats received their injection of the training dose of *R*(−)-MDA or *S*(+)-MDA. As shown in figures 11 and 12, pirenperone is a potent antagonist ($AD_{50}$ = 15 μg/kg) of the *R*(−)-MDA stimulus but had no effect on *S*(+)-MDA lever appropriate responding. These results are consistent with those reported by Broadbent *et al.* (1992), with separate groups of rats trained to discriminate 1.25 mg/kg of either *R*(−)-MDA or *S*(+)-MDA from saline, and suggest a 5-$HT_{2A/2C}$ component in the mechanism of action of *R*(−)-MDA. In this regard, it should be noted that recent studies (Ismaiel *et al.*, 1993; Schrieber *et al.*, 1994) have reported on the selective 5-$HT_{2A}$ agents AMI-193 (8-(3-(4-fluorophenoxy)propyl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one) and MDL 100,907 (*R*-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)-4-piperidinemethanol], respectively. AMI-193 and MDL 100,907 display a 2000-fold and a 200-fold selectivity for 5-$HT_{2A}$ *versus* 5-$HT_{2C}$ receptors, respectively. These investigators also reported that AMI-193 is a very potent antagonist

($AD_{50}$ = 3 μg/kg) of a 1.0 mg/kg DOM stimulus in rats and MDL 100,907 is an extremely potent antagonist ($AD_{50}$ = 0.6 μg/kg) of a 0.63 mg/kg DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane) stimulus in rats. Their data seem to indicate that the DOM and DOI discriminative stimuli are mediated primarily by 5-$HT_{2A}$ receptors. Thus, it might be speculated that *R*(−)-MDA's discriminative stimulus effect could be mediated, at least in part, by agonist action at 5-$HT_{2A}$ receptors and that these new antagonists would be very effective in blocking *R*(−)-MDA's stimulus effect.

In summary, this study is the first demonstration that optical isomers of the same compound can be differentiated using a three-lever drug discrimination task. Moreover, the following points support the contention that each enantiomer of MDA can produce a markedly different stereoselective stimulus effect: 1) rats can be trained to discriminate the two isomers of MDA, 2) the animals respond primarily on the *R*(−)-MDA-appropriate lever after DOM, *S*(+)-DOM and mescaline injection, and respond primarily on the *S*(+)-MDA-appropriate lever after *S*(+)-AMPH and cocaine administration and 3) the animals respond on the saline-designated lever after the combination injection of pirenperone and *R*(−)-MDA, but respond on the *S*(+)-MDA-designated lever after the combination injection of pirenperone and *S*(+)-MDA.

### References


Broadbent, J., Appel, J. B., Michael, E. K. and Ricker, J. H.: Discriminative stimulus effects of the optical isomers of 3,4-methylenedioxyamphetamine (MDA). Behav. Pharmacol. **3:** 443–454, 1992.

Broadbent, J., Michael, E. K. and Appel, J. B.: Generalization of cocaine to the isomers of 3,4-methlenedioxyamphetamine and 3,4-methylenedioxymethamphetamine. Drug Dev. Res. **16:** 443–450, 1989.

Callahan, P. M. and Appel, J. B: Differences in the stimulus properties of 3,4-methylendioxyamphetamine and 3,4-methylenedioxymethamphetamine in animals trained to discriminate hallucinogens from saline. J. Pharmacol. Exp. Ther. **246:** 866–870, 1988.

Colpaert, J. C., Niemegeer, C. J. E. and Janssen, P. A. J.: A drug discrimination analysis of lysergic acid diethylamide (LSD): *In vivo* agonist and antagonist effects of purported 5-hydroxytryptamine antagonists and of pirenperone, a LSD antagonist. J. Pharmacol. Exp. Ther. **221:** 206–214, 1982.

Evans, S. M. and Johanson, C. E.: Discriminative stimulus properties of 3,4-methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine in pigeons. Drug Alcohol Depend. **18:** 159–164, 1986.

Finney, D. J.: Statistical Methods in Biology Assay, 2nd ed., Hafner, New York, 1964.

Glennon, R. A.: Stimulus properties of hallucinogenic phenalkylamines and related designer drugs: Formulation of structure activity relationships. In Pharmacology and toxicology of amphetamine and related designer drugs, ed. by K. Ashgar and E. De Souza, pp. 43–67, NIDA Research Monograph 94, Washington, DC, U.S. Government Printing Office, 1989.

Glennon, R. A. and Hauck, A. E.: Mechanistic studies on DOM as a discriminative stimulus. Pharmacol. Biochem. Behav. **23:** 937–941, 1985.

Glennon, R. A. and Young, R.: MDA: A psychoactive agent with dual stimulus effects. Life Sci. **34:** 379–383, 1984a.

Glennon, R. A. and Young, R.: MDA: An agent that produces stimulus effects similar to those of 3,4 DMA, LSD and cocaine. Eur. J. Phamacol. **99:** 249–250, 1984b.

Glennon, R. A. and Young, R.: Further investigation of the discriminative stimulus properties of MDA. Pharmacol. Biochem. Behav. **20:** 501–505, 1984c.

Glennon, R. A., Rosecrans, J. A. and Young, R.: Drug-induced discrimination: A description of the paradigm and a review of its specific application to the study of hallucinogenic agents. Med. Res. Rev. **3:** 289–340, 1983a.

Glennon, R. A., Young, R. and Rosecrans, J. A.: Antagonism of the effects of the hallucinogen DOM and the purported 5-HT agonist quipazine by 5-HT2 antagonists. Eur. J. Pharmacol. **91:** 189–196, 1983b.

Glennon, R. A., Young, R., Rosecrans, J. A. and Anderson, G. M.: Discriminative stimulus properties of MDA analogs. Biol. Psychiatry **17:** 807–814, 1982.

Hartig, P. R.: Molecular biology of 5-HT receptors. Trends Pharmacol. Sci. **10:** 64–69, 1989.

Ismaiel, A. M., De Los Angeles, J., Teitler, M., Ingher, S. and Glennon, R. A.: Antagonism of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane stimulus with a newly identified 5-HT2- *versus* 5-HT1C-selective antagonist. J. Med. Chem. **36:** 2519–2525, 1993.

Kamien, J. B., Johanson, C. E., Schuster, C. R. and Woolverton, W. L.: The effects of (±) methylenedioxymethamphetamine and (±) methylenedioxyamphetamine in monkeys trained to discriminate (+) amphetamine from saline. Drug Alcohol Depend. **18:** 139–147, 1986.

Oberlender, R. and Nichols, D. E.: Drug discrimination studies with MDMA and amphetamine. Psychopharmacology **95:** 71–76, 1988.

Schreiber, R., Brocco, M. and Millan, M. J.: Blockade of the discriminative stimulus effects of DOI by MDL 100,907 and the "atypical" antipsychotics, clozapine and risperidone. Eur. J. Pharmacol. **264:** 99–102, 1994.

Shulgin, A. T.: Psychotominetic drugs: Structure-activity relationships. *In* Handbook of Psychopharmacology, vol. 11, ed. by L. L. Iversen, S. D. Iversen and S. H. Snyder, p. 243, Plenum Press, New York, 1978.

Silverman, P. B. and Ho, B. T.: The discriminative stimulus properties of 2,5 dimethoxy-4-methylamphetamine (DOM): Differentiation from amphetamine. Psychopharmacology **68:** 209–215, 1980.

Terry, P., Witkin, J. M. and Katz, J. L.: Pharmacological characterization of the novel discriminative stimulus effects of a low dose of cocaine. J. Pharmacol. Exp. Ther. **270:** 1041–1048, 1994.

Tilson, H. A., Baker, T. G. and Gylys, J. A.: A comparison of the discriminative stimulus properties of R-2,5-dimethoxy-4-methylamphetamine (R-DOM) and S-amphetamine in the rat. Psychopharmacologia **44:** 225–228, 1975.

White, F. J. and Appel, J. B.: Training dose as a factor in LSD-saline discrimination. Psychopharmacolgy **76:** 20–25, 1982.

Young, R. and Glennon, R. A.: Discriminative stimulus properties of amphetamine and structurally related phenalkylamines. Med. Res. Rev. **6:** 99–130, 1986.

Young, R., Glennon, R. A. and Rosecrans, J. A.: Discriminative stimulus properties of the hallucinogenic agent DOM. Commun. Psychopharmacol. **4:** 501–506, 1980.


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