Time Course Analysis of the Discriminative Stimulus Effects of the Optical Isomers of 3,4-Methylenedioxymethamphetamine (MDMA)

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BAKER, L. E., T. B. VIRDEN, M. E. MILLER AND C. L. SULLIVAN. *Time course analysis of the discriminative stimulus effects of the optical isomers of 3,4-methylenedioxymethamphetamine (MDMA).* PHARMACOL BIOCHEM BEHAV **58**(2) 505–516, 1997.—The present study examined the discriminative stimulus effects of the MDMA optical isomers administered at different presession injection intervals. In the first experiment, male Sprague-Dawley rats were trained in a two-lever, food-reinforced operant procedure to discriminate either (+)-MDMA (1.25 mg/kg) or (−)-MDMA (3.50 mg kg) at either 20 or 90 min following injection. Animals administered (+)-MDMA or saline 90 min before training sessions failed to attain the discrimination criteria after 73 training sessions, whereas (−)-MDMA successfully established discriminative stimulus control at both the 20 min and the 90 min postinjection intervals. (+)-Amphetamine did not substitute for either isomer, although a significant amount of drug-appropriate responding occurred in animals trained to discriminate (+)-MDMA at 20 min and (−)-MDMA at 90 min. Sch 39166 partially reduced the discrimination of (+)-MDMA at 20 min and (−)-MDMA at 90 min, although this effect was not dose dependent. Sch 39166 had no effect on animals trained to discriminate (−)-MDMA at 20 min. Haloperidol did not alter the discrimination of (+)-MDMA at 20 min but partially reduced the discriminative stimulus control of (−)-MDMA at 20 min and (−)-MDMA at 90 min. Fenfluramine substituted for both isomers of MDMA. Pirenpirone completely blocked the discriminative stimulus effects of (−)-MDMA at 20 min, although (+)-MDMA at 20 min and (−)-MDMA at 90 min were only partly blocked. WAY 100,135 had little effect on drug-appropriate responding; however, the discrimination of (+)-MDMA at 20 min was partly reduced by this 5-HT$_1$A antagonist. In a second experiment, rats trained to discriminate (+)-MDMA (1.5 mg/kg) or (−)-MDMA (3.0 mg/kg) from saline were administered substitution tests with both isomers 20, 60, 90 and 120 min after injection. Results confirmed those of the first experiment that (+)-MDMA appears to have a shorter duration of action than (−)-MDMA. These results are discussed in light of the training doses employed. © 1997 Elsevier Science Inc.

MDMA isomers   Amphetamine   Serotonin   Dopamine   Drug discrimination   Rats

THE ring-substituted phenylisopropylamine 3,4-methylene-dioxymethamphetamine (MDMA, "Ecstasy") is a popular drug of abuse that is commonly reported to intensify affect, enhance self-awareness and facilitate communication and intimacy among users (1,7,23,29). Although MDMA has been compared with both hallucinogens and psychostimulants, some researchers have suggested a distinct classification for MDMA (e.g., "entactogens") (20,21). Despite its structural similarity to amphetamine, investigations in nonhuman animals have indicated that MDMA is not simply an amphetamine-like compound. Although the neurochemical actions of amphetamine are mediated primarily through dopamine release, MDMA is a potent serotonin (5-HT) releaser (19). MDMA also facilitates dopamine (DA) release (17), and this appears to be mediated primarily through 5-HT$_2$ receptor activation (28). However, 5-HT release seems to be a critical component of the complex discriminative stimulus effects of MDMA (2,15,26), whereas DA release may play only a minor role in these effects. In contrast, the discriminative stimulus effects of amphetamine are primarily dopaminergically mediated (27).

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Several investigations have revealed that MDMA produces compound discriminative stimulus effects, and an amphetamine-like effect may be only a partial component of these effects (2,9,15,26). In pigeons (8) and rats (14), MDMA substituted for (+)-amphetamine, although other investigators (22) have not confirmed these findings in rats. Furthermore, reciprocal substitution does not occur between MDMA and (+)-amphetamine. That is, (+)-amphetamine does not substitute in animals trained to discriminate MDMA (3,11,26). In addition, MDMA substitutes for the serotonin releaser, fenfluramine (24), a substance that produces subjective effects unlike (+)-amphetamine in humans (6) and non-humans (9). Other serotonergic agents such as norfenfluramine and N-(3-Trifluoromethylphenyl)piperazine (TFMPP) also substitute for MDMA (26).

Drug discrimination investigations of the optical isomers of MDMA suggest that these enantiomers may produce distinct psychoactive effects and that (+)-MDMA may be more like amphetamine than (−)-MDMA. Glennon et al. (14) reported that (+)-MDMA but not (−)-MDMA substituted for (+)-amphetamine, although Oberlender and Nichols (22) found neither isomer to substitute for (+)-amphetamine. Furthermore, (+)-amphetamine engendered very little drug-appropriate responding in animals trained to discriminate either (+)-MDMA or (−)-MDMA (2). In contrast, fenfluramine and another potent 5-HT releaser, p-chloroamphetamine substituted for both isomers of MDMA (2). Other serotonergic compounds with known hallucinogenic effects have also been compared with the optical isomers of MDMA. Neither isomer substituted for the hallucinogen 2,5-dimethoxy-4-methylphenylisopropylamine (DOM) (12) or LSD (5), although both isomers substituted for mescaline (5). However, mescaline produced very little drug-appropriate responding in animals trained to discriminate either MDMA isomer, whereas DOM substituted partially for (−)-MDMA but not (+)-MDMA (2). The lack of cross substitution between these hallucinogens and the optical isomers of MDMA also supports the contention that MDMA produces a more complex profile of discriminative stimulus effects.

Because serotonin releasers substitute more completely and reliably for MDMA and its optical isomers than substances with primarily dopaminergic actions, the discriminative stimulus properties of MDMA seem to be mediated primarily by serotonergic mechanisms. However, dopamine-mediated effects may play a weak but significant role, particularly during longer postinjection intervals (26). Schechter (26) investigated the discriminative stimulus properties of racemic MDMA at two presession injection intervals (20 and 105 min) and found that the dopamine antagonist haloperidol had little effect on drug appropriate responding in animals trained to discriminate MDMA at the 20-min interval but significantly reduced drug-appropriate responding in animals trained at the 105-min interval. These data suggest the presence of a dopaminergic component, albeit weak, in mediating the discriminative stimulus properties of MDMA. The 5-HT antagonist pirenpirone antagonized the discriminative stimulus effects of MDMA at both time intervals equally (26). However, because that study employed a different training dose at each time interval, the results are somewhat ambiguous as to whether the different degree of antagonism with haloperidol was due to a dose effect or a time effect. The initial purpose of the present study was to reexamine serotonergic and dopaminergic mechanisms underlying the discriminative stimulus effects of each MDMA isomer in animals trained to discriminate these drugs following different presession injection intervals. In the first experiment, rats were trained to discriminate either (+)-MDMA or (−)-MDMA from saline in training sessions that started either 20 min or 90 min after injection, and tests of stimulus generalization and antagonism were administered with dopaminergic and serotonergic agents. Results suggested that (+)-MDMA (1.25 mg/kg) did not establish discriminative stimulus control when injected 90 min prior to training sessions. Thus, a second experiment was conducted to examine further the time course of each enantiomer of MDMA. In the second experiment, rats were trained to discriminate either (+)-MDMA or (−)-MDMA from saline 20 min after injection and were administered substitution tests with these isomers 20, 60, 90 and 120 min after injection.

EXPERIMENT 1

Materials and Methods

Subjects. Subjects were experimentally naive, male Sprague-Dawley rats (Harlan Breeding Laboratories, Indianapolis, IN), aged approximately 60 days at the beginning of the study. Animals were housed individually in wire-mesh cages in a colony maintained on a 12-h light (0700–1900)/12-h dark cycle and at a constant temperature (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 a 28-v house light and a dipper (0.1 ml) mounted equidistant between two levers. A Zenith 320-SX computer was programmed with MED-PC instrumentation and software (MED Associates Inc.; version 2.0) to control experimental events and data collection.

Drugs. (+)-MDMA-HCl, (−)-MDMA-HCl, (+)-amphetamine-sulfate and (+)-fenfluramine-HCl were supplied by the National Institute on Drug Abuse (Rockville, MD). The D1 antagonist, Sch 39166-HCl, was generously provided by the Schering-Plough Corporation (Bloomfield, NJ). The 5-HT$_{1A}$ antagonist, WAY 100,135 was provided by Pharmacia & Upjohn, Inc. (Kalamazoo, MI), with permission from Wyeth Aerst, Inc. to synthesize this compound. The 5-HT$_2$ antagonist, pirenpirone, was purchased from Research Biochemicals, Inc. (Natick, MA), and the D2 antagonist, haloperidol, was purchased from Sigma Chemical Company (St. Louis, MO). Where indicated, drug doses refer to the salt form of the drug. With the exception of WAY 100,135, pirenpirone and haloperidol, drugs were dissolved in 0.85% physiological saline. Haloperidol and pirenpirone were dissolved in distilled water with a few drops of 0.1 N HCl, and WAY 100,135 was suspended in 0.3% methyl cellulose. Sch 39166 and WAY 100,135 were administered subcutaneously (SC). All other drugs were administered intraperitoneally (IP).

Discrimination training. Sixteen rats were trained to discriminate (+)-MDMA (1.25 mg/kg) and 16 were trained to discriminate (−)-MDMA (3.5 mg/kg) from saline in a two-choice operant task under a fixed ratio 20 (FR 20) schedule of reinforcement. Training doses were chosen based on previous experiments conducted in a different laboratory (2). Eight animals assigned to each training dose were injected IP 20 min prior to each training session, and the remaining animals received IP injections 90 min prior to training sessions. For half the animals in each group of eight, responding on the right lever was reinforced with sweetened condensed milk (1 part

milk: 2 parts tap water) after drug injections, and responding on the left lever was reinforced following saline injections; conditions were reversed for the remaining animals. To reduce olfactory stimuli (10), levers were wiped with isopropyl alcohol before each session.

Training sessions lasted 20 min and were conducted 6 days each week (Monday–Saturday) at approximately the same time each day. The first two training sessions were conducted under saline-appropriate conditions; thereafter, drug and saline conditions were presented in a pseudorandom order, such that neither condition was presented for more than two consecutive sessions. Thus, on average, each condition was presented three times each week. Training under each condition began under a FR 1 schedule, and when responding was stable, the number of consecutive correct responses required for reinforcement was gradually increased from 1 to 20. Responses on the incorrect lever reset the response counter, and no reinforcement was delivered until 20 consecutive responses were made on the correct lever. When animals attained accuracies of at least 80% correct (prior to delivery of the first reinforcer) for a minimum of 9 of 10 consecutive training sessions, testing began.

Stimulus substitution testing. Substitution tests were conducted with lower doses of (+)-MDMA (0.312–1.25 mg/kg) in the rats trained to discriminate (+)-MDMA 20 min following injection. Tests were not conducted with the (+)-MDMA 90-min group because the discrimination criterion was not met

by these subjects (see Results). Rats trained to discriminate (−)-MDMA 20 min after injection were administered substitution tests with lower doses of this isomer (0.75–3.5 mg/kg) 20 min after injection, and rats trained to discriminate (−)-MDMA 90 min after injection were tested with these doses 90 min after injection. In all three training groups that met the discrimination criterion, substitution tests were also administered with (+)-amphetamine (0.25–1.5 mg/kg; 15 min, IP) and (+)-fenfluramine (0.5–4 mg/kg; 15 min, IP). The order of test doses was randomized and counterbalanced across subjects in each training group. For each drug, substitution tests were administered every other day, and maintenance training sessions were conducted between test days. Each test dose was examined twice in each animal, once following a drug training session and once following a saline training session, in subjects that maintained the discrimination criterion during training sessions. After all doses of a particular drug were tested, subjects were trained for a minimum of 1 week without additional testing before the next dose–effect relationship was examined. During test sessions, animals were injected with the test solution and placed in the chambers for 20 min or until 20 consecutive responses were made on either lever. Lever pressing was not reinforced during test sessions, and animals were immediately removed from the chambers when the test was completed.

Antagonist testing. Antagonist tests were conducted with the DA antagonists Sch 39166 (0.025–0.1 mg/kg; 30 min, SC)
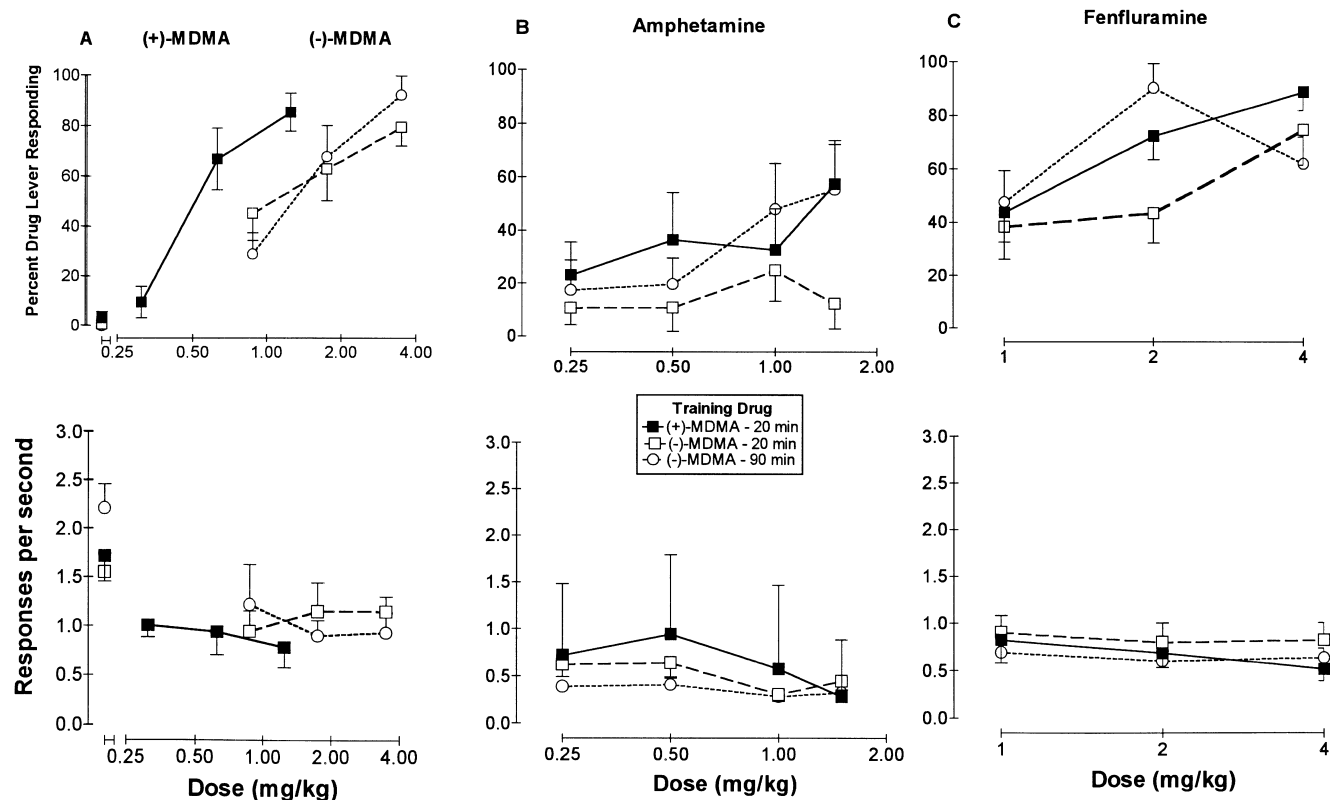

FIG. 1. A: Dose–response functions for each training drug. Dose–response functions for (+)-amphetamine (B) and (+)-fenfluramine (C) in rats trained to discriminate (+)-MDMA at 20 min. (−)-MDMA at 20 min. (−)-MDMA at 90 min. Percentage of total responses on the training drug lever is shown above and response rate is shown below. The symbols next to the Y-axis in A indicate data from saline control tests. Each data point represents a mean from eight animals.

TABLE 1

ANOVA RESULTS FOR EXPERIMENTS 1 AND 2

Experiment 1		
Drug	Percentage of Drug-Lever	Response Rate
(+)-Amphetamine		
Dose	$F(4,98)=5.26**$	$F(4,98)=48.18***$
Training group	$F(2,98)=3.76*$	NS
Dose × training group	NS	NS
(+)-Fenfluramine		
Dose	$F(4,100)=30.54***$	$F(4,100)=14.78***$
Training group	NS	NS
Dose × training group	NS	NS
Sch 39166		
Dose	$F(3,69)=4.80**$	$F(3,69)=3.25*$
Treatment	NS	NS
Dose × training group	NS	NS
Haloperidol		
Dose	$F(3,66)=3.83*$	$F(3,66)=7.15**$
Treatment	NS	NS
Dose × training group	NS	NS
Pirenpirone		
Dose	$F(3,68)=20.25***$	$F(3,67)=11.69***$
Treatment	NS	NS
Dose × training group	$F(6,68)=2.58*$	NS
WAY 100,135		
Dose	$F(3,84)=6.16**$	$F(3,83)=2.93*$
Treatment	NS	$F(2,83)=3.33*$
Dose × training group	NS	NS

	Experiment 2			
	(+)-MDMA % Drug Lever	Response Rate	(−)-MDMA % Drug Lever	Response Rate
Test drug	NS	NS	$F(1,59) = 10.54**$	NS
Dose	$F(4,40) = 12.09***$	$F(4,40) = 4.21**$	$F(4,59) = 29.61***$	$F(4,60) = 8.14***$
Test drug × dose	NS	NS	$F(4,59) = 3.50*$	NS
Test drug	$F(2,44) = 12.57***$	$F(2,44) = 11.80***$	$F(2,64) = 50.00***$	$F(2,64) = 8.38**$
Time	$F(3,44) = 3.39*$	NS	$F(3,64) = 21.36***$	NS
Test drug × time	$F(6,44) = 2.38*$	NS	$F(6,64) = 9.26***$	NS

$*p < 0.05.$ $**p < 0.005.$ $***p < 0.0001.$
NS = not significant.

and haloperidol (0.125–0.5 mg/kg; 60 min, IP) and with the 5-HT antagonists WAY 100,135 (2.5–10.0 mg/kg; 45 min, SC) and pirenpirone (0.16–0.64 mg/kg; 60 min, IP). These compounds were administered in combination with the training compound given at the appropriate time interval. Test sessions were conducted in a manner similar to that described for the substitution tests. However, a full week of training did not occur for all animals between dose effect determinations for pirenpirone and WAY 100,135.

Data analysis. Substitution and antagonism data were presented as the mean percentage of total responses made on the drug-appropriate lever during test sessions. Response rate was indicated as the mean number of responses made (on either lever) per second during test sessions. Data from animals that did not complete at least 20 total responses during test sessions were not included in the analyses. A particular dose of a test compound was considered to have substituted for the training drug if the mean percentage of drug-appropriate responding was 80% or greater. A particular dose of a test compound was considered to have completely blocked the stimulus effects of the training compound if the mean percentage of drug-appropriate responding was less than 20%. The results of substitution tests with (+)-MDMA were analyzed with a one-factor repeated measures analysis of variance (ANOVA). The results of substitution tests with (−)-MDMA were analyzed with a two-factor (dose and presession injection interval) ANOVA. The results of other substitution tests and antagonism tests were analyzed with a two-factor mixed ANOVA with treatment (training group) as a between-subjects factor and dose as a within-subjects factor. Separate analyses were conducted on the percentage of drug-lever response data and the response rate data. For drugs that pro-

TABLE 2

PERCENT OF DRUG LEVER RESPONDING FOLLOWING ADMINISTRATION OF DA AND 5-HT ANTAGONISTS
IN COMBINATION WITH (+)-MDMA AT 20 MIN, (−)-MDMA AT 20 MIN OR (−)-MDMA AT 90 MIN

	(+)-MDMA at 20 min		(−)-MDMA at 20 min		(−)-MDMA at 90 min	
Test Drug Dose	% Drug Mean (SEM)	n/N	% Drug Mean (SEM)	n/N	% Drug Mean (SEM)	n/N
Saline + training drug	100 (0.0)	8/8	93 (6.2)	8/8	93 (7.1)	7/8
Sch 39166						
0.025	72 (14.3)	8/8	94 (6.2)	8/8	85 (9.8)	7/8
0.05	50 (13.4)	8/8	92 (7.0)	7/8	50 (18.2)	6/8
0.10	96 (2.7)	6/8	88 (12.5)	4/8	75 (25)	4/8
Haloperidol						
0.125	74 (8.3)	8/8	92 (7.1)	7/8	97 (3.0)	6/8
0.25	83 (11.3)	6/8	80 (14.3)	7/8	59 (24.1)	5/8
0.50	76 (19)	5/8	58 (16.6)	6/8	92 (7.6)	5/8
Pirenpirone						
0.16	38 (11.4)	8/8	76 (13.6)	7/8	41 (12.1)	8/8
0.32	29 (11.6)	8/8	74 (13.1)	7/8	34 (9.6)	7/8
0.48	100 (0)	1/3	31 (13.3)	6/6	31 (0.1)	3/4
0.64	60 (40)	2/8	14 (12.2)	4/8	29 (19.8)	5/8
WAY 100,135						
2.5	87 (7.7)	8/8	74 (0.2)	8/8	99 (1.1)	8/8
5.0	74 (13.4)	8/8	79 (8.3)	8/8	83 (9.9)	8/8
10.0	55 (8.1)	8/8	78 (9.9)	8/8	83 (12.2)	8/8

duced substitution for or antagonism of either training drug, ED_{50}s were calculated. The statistical analyses and ED_{50} calculations were performed with the statistical software Graph-Pad Prism (GraphPad, Inc., San Diego, CA).

RESULTS

Animals administered (+)-MDMA or saline 20 min prior to training sessions attained the discrimination criterion within a mean of 75 training sessions (range = 64–93), whereas animals administered (−)-MDMA or saline 20 min prior to training sessions acquired the discrimination criterion within a mean of 65 training sessions (range = 39–76). Animals administered (+)-MDMA or saline at a 90-min presession injection interval failed to attain the discrimination criterion, thus, substitution and antagonism tests were not conducted in this group of subjects. In contrast, the group that was administered (−)-MDMA or saline 90 min prior to training sessions acquired the discrimination criterion within a mean of 72 training sessions (range = 66–88).

Figure 1A illustrates the dose–response curves with the training drugs that were determined in the three groups in which the discrimination criterion was met. Approximately 60% of drug-appropriate responding was observed with 0.625 mg/kg (+)-MDMA and 1.75 mg/kg (−)-MDMA. Lower doses of each isomer produced little drug-appropriate responding. The ED_{50} for (+)-MDMA at 20 min was 0.59 mg/kg. The dose–response functions for (−)-MDMA administered at a 20-min presession interval (ED_{50} = 1.02 mg/kg) and this isomer administered at a 90-min interval (ED_{50} = 1.32 mg/kg) were very similar. One-factor repeated measures ANOVAs on the results of substitution tests with (+)-MDMA showed a significant dose effect on percentage of drug-lever responding [$F(3,31) = 25.27, p < 0.001$] and response rate [$F(3,31) = 7.07$, $p < 0.0005$]. Two-factor ANOVAs on the results of (−)-MDMA substitution tests only showed a significant main ef-

fect of dose on percentage of drug-lever responding [$F(3,54) = 32.82, p < 0.0001$] and response rate [$F(3,54) = 5.49, p < 0.005$].

Results of statistical analyses of the data from substitution and antagonism tests are summarized in Table 1. Figure 1B shows that (+)-amphetamine did not substitute for either isomer of MDMA. The 1.5-mg/kg dose of (+)-amphetamine produced less than 20% drug-appropriate responding in rats trained to discriminate (−)-MDMA at 20 min. In contrast, this dose produced approximately 60% drug-appropriate responding in rats trained to discriminate (−)-MDMA at 90 min and in rats trained to discriminate (+)-MDMA at 20 min. A two-factor mixed ANOVA revealed significant main effects of dose and treatment (training drug) on percentage of drug-lever responding produced by (+)-amphetamine. Only the main effect of dose was significant on response rate (see Table 1).

Figure 1C illustrates the dose–response functions determined with (+)-fenfluramine in each of the three training groups. This 5-HT releaser substituted at a dose of 4 mg/kg for (+)-MDMA at 20 min (ED_{50} = 1.09 mg/kg) and at a dose of 2 mg/kg for (−)-MDMA at 90 min (ED_{50} = 1.0 mg/kg). Although 4.0 mg/kg (+)-fenfluramine substituted completely (≥97%) in five of the eight animals in the (−)-MDMA-at-20-min training group, the mean for the group was only 75% at this dose and the ED_{50} for fenfluramine substitution was slightly higher in this training group (1.42 mg/kg). Only the main effect of dose was significant on both percent drug-lever responding and response rate (see Table 1).

Table 2 summarizes the results of the antagonist tests with Sch 39166, haloperidol, pirenpirone and WAY 100,135 on percentage of drug-lever responding in each of the three training groups. The effects of these drugs on response rate are summarized in Table 3. Sch 39166 (0.05 mg/kg) reduced drug-appropriate responding to 50% in rats trained to discriminate (+)-MDMA at 20 min and (−)-MDMA at 90 min. However, this effect was not dose dependent; 0.1 mg/kg did not further reduce drug-appropriate responding. However,

TABLE 3

RESPONSE RATE DURING TESTS WITH DA AND 5-HT ANTAGONISTS IN COMBINATION
WITH (+)-MDMA AT 20 MIN, (−)-MDMA AT 20 MIN OR (−)-MDMA AT 90 MIN

	(+)-MDMA at 20 min		(−)-MDMA at 20 min		(−)-MDMA at 90 min	
Test Drug Dose	Responses per second Mean (SEM)	n/N	Responses per second Mean (SEM)	n/N	Responses per second Mean (SEM)	n/N
Saline + training drug	1.02 (0.17)	8/8	0.76 (0.13)	8/8	1.11 (0.17)	7/8
Sch 39166						
0.025	1.08 (0.21)	8/8	0.96 (0.19)	8/8	0.75 (0.16)	7/8
0.05	0.66 (0.23)	8/8	0.48 (0.22)	7/8	0.81 (0.12)	6/8
0.10	0.50 (0.28)	6/8	0.30 (0.24)	4/8	0.59 (0.54)	4/8
Haloperidol						
0.125	0.67 (0.13)	8/8	0.90 (0.28)	7/8	0.88 (0.18)	6/8
0.25	0.64 (0.33)	6/8	0.50 (0.22)	7/8	0.45 (0.12)	5/8
0.50	0.23 (0.11)	5/8	0.31 (0.07)	6/8	0.34 (0.09)	5/8
Pirenpirone						
0.16	0.51 (0.11)	8/8	0.38 (0.06)	7/8	0.60 (0.14)	8/8
0.32	0.46 (0.17)	8/8	0.28 (0.07)	7/8	0.39 (0.13)	7/8
0.48	0.30 (0.00)	1/3	0.31 (0.14)	6/6	0.44 (0.07)	3/4
0.64	0.28 (0.12)	2/8	0.53 (0.07)	4/8	0.39 (0.15)	5/8
WAY 100,135						
2.5	0.66 (0.13)	8/8	0.57 (0.23)	8/8	0.52 (0.12)	8/8
5.0	1.06 (0.26)	8/8	0.44 (0.15)	8/8	0.69 (0.17)	8/8
10.0	0.81 (0.19)	8/8	0.56 (0.15)	8/8	0.49 (0.10)	8/8

the 0.1-mg/kg dose severely disrupted responding in half the subjects trained to discriminate (−)-MDMA at either interval. Only four of the eight animals in both groups of (−)-MDMA-trained rats completed 20 responses during tests with this dose of Sch 39166. On percentage of drug-lever responding and response rate, only the main effect of dose was significant (see Table 1).

The D2 antagonist haloperidol reduced (−)-MDMA-appropriate responding to about 60% in rats trained to discriminate this isomer at either injection interval, but this effect was not dose-dependent in the rats trained to discriminate (−)-MDMA at 90 min. Haloperidol had little effect on drug-appropriate responding in animals trained to discriminate (+)-MDMA at 20 min. Again, only the main effect of dose was significant on both percentage of drug-lever responding and response rate (see Table 1).

The 5-HT$_2$ antagonist pirenpirone reduced drug-appropriate responding to about 30% when administered in combination with (+)-MDMA at 20 min and in combination with (−)-MDMA at 90 min. The highest dose (0.64 mg/kg) severely disrupted responding in the (+)-MDMA-20-min-trained animals; only two of the eight rats in this group completed at least 20 responses during test sessions with the 0.64-mg/kg dose. Because this dose was highly disruptive, a dose of 0.48 mg/kg was tested in some of the animals. This dose was also highly disruptive. In animals trained to discriminate (−)-MDMA at 20 min, pirenpirone (0.64 mg/kg) reduced drug-lever responding to 14%. Statistical analyses revealed a significant main effect of dose and a significant dose × treatment interaction on percentage of drug-lever responding (Table 1). The dose-dependent effects of pirenpirone on response rate were also significant (Table 1).

The 5-HT$_{1A}$ antagonist, WAY 100,135 (10 mg/kg) reduced drug-appropriate responding to 55% in rats trained to discriminate (+)-MDMA at 20 min but had little effect in rats trained to discriminate (−)-MDMA at either presession injec-

tion interval. Only the main effect of dose on percentage of drug-lever responding was significant (Table 1). The main effects of dose and treatment were significant on response rate (Table 1).

EXPERIMENT 2

Because (+)-MDMA did not successfully establish discriminative stimulus control when administered 90 min prior to training sessions, a second experiment was conducted to examine further the differences in the time course of the two MDMA isomers. Rats were trained to discriminate either (+)-MDMA or (−)-MDMA from saline, and substitution tests were administered with the training drugs at different presession injection intervals. So that comparisons could be made more easily between the dose effect functions of each isomer, slightly different training doses were used in the second experiment. That is, 1.5 mg/kg (+)-MDMA and 3.0 mg/kg (−)-MDMA were used, so that the training doses differed by a simple factor of 2. The only other methodological difference between experiments 1 and 2 was the type of reinforcer used. For practical reasons, water rather than milk was used as a reinforcer in experiment 2.

Materials and methods

Subjects. Subjects were 16 experimentally naive, male Sprague-Dawley rats (Harlan Breeding Laboratories) aged approximately 60 days and weighing 270–325 g at the beginning of the study. Animals were housed individually in a manner similar to that in experiment 1. Standard laboratory rodent diet was available ad libitum. Access to water was restricted to amounts obtained during training sessions, for 10–15 min following training and test sessions and for at least 24 h on weekends.

Apparatus and drugs. The apparatus was the same as that used in experiment 1. The isomers of MDMA (National Insti-
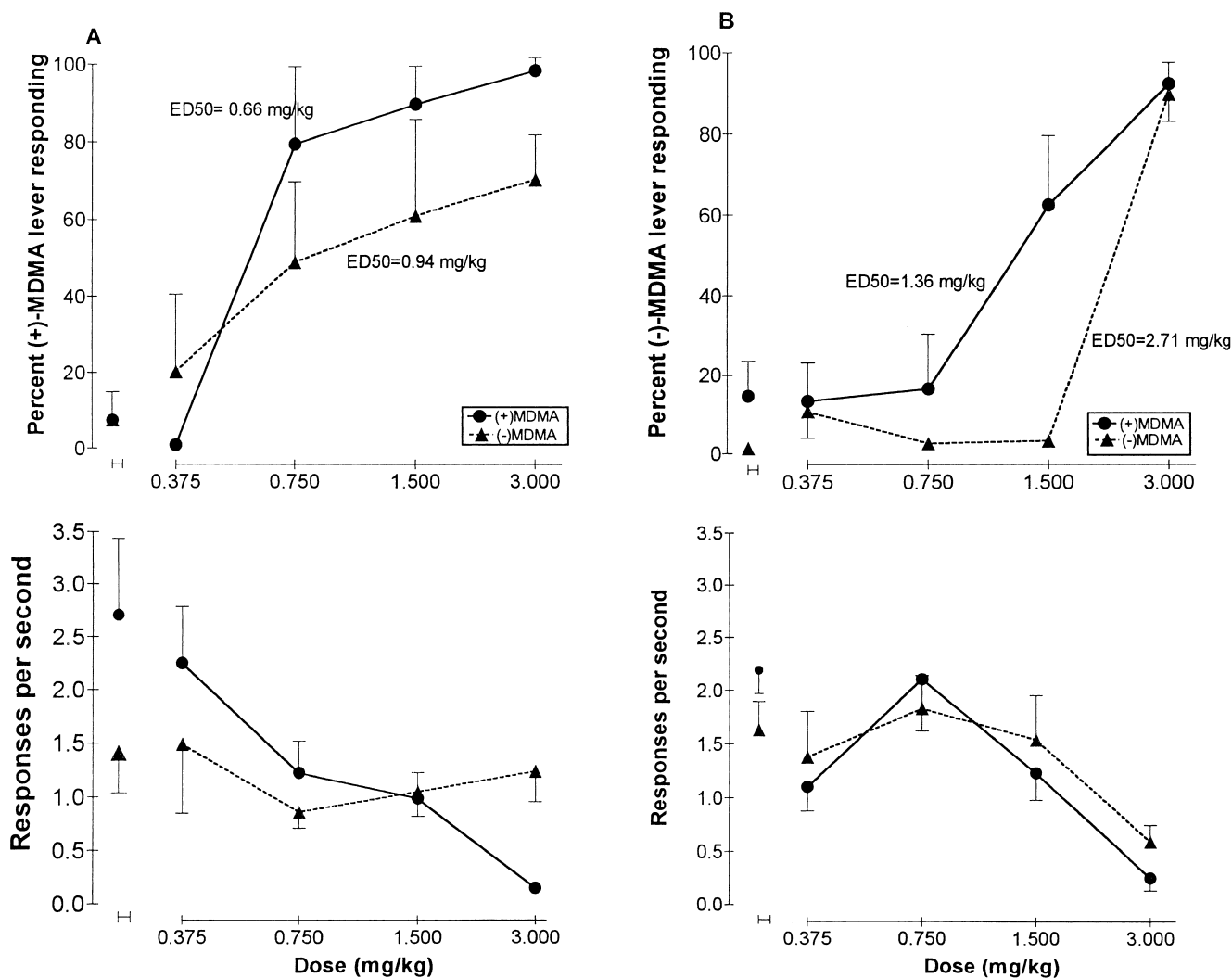
FIG. 2. Dose–response functions for (+)-MDMA and (−)-MDMA in animals trained to discriminate (+)-MDMA 1.5 mg/kg ($n = 5$; A) and (−)-MDMA 3.0 mg/kg ($n = 7$; B). Percentage of total responses on the training drug lever is shown above and response rate is shown below. The symbols located next to the Y-axis indicate data from saline control tests.

tute on Drug Abuse) were administered using slightly different doses from those used in experiment 1 but were otherwise prepared and injected in a similar manner.

Discrimination training. Animals were trained to discriminate (+)-MDMA (1.5 mg/kg; $n = 8$) or (−)-MDMA (3.0 mg/kg; $n = 8$) from saline in a two-lever, water-reinforced drug-discrimination task. With the exception of reinforcer type, discrimination training procedures were conducted in a manner consistent with those described in experiment 1. When subjects attained accuracies of at least 85% correct (prior to delivery of the first reinforcer) for a minimum of 9 of 10 consecutive training sessions, testing began.

Stimulus substitution testing. Animals were administered substitution tests with several doses of each isomer of MDMA (0.375, 0.75, 1.5 and 3.0 mg/kg). In addition, the 1.5 and 3.0 mg/kg doses of each MDMA isomer were administered at several different presession intervals (20, 60, 90, 120 min) and tested for stimulus generalization. Test sessions were conducted once

or twice a week in animals that maintained a minimum of 80% condition-appropriate responding for at least two consecutive training sessions (i.e., drug and saline) between tests.

Data analysis. For each test, the percentage of total responses on the drug-appropriate lever was calculated for each subject. Response rate was expressed as the number of total responses (on both levers) per second. Means were calculated for each training group and plotted for visual analysis. The data from subjects that emitted fewer than 20 total responses during a test session were excluded from the analyses. A particular dose of a test compound was considered to have substituted for the training drug if the mean percentage of drug-appropriate responding was 80% or greater. Drug-appropriate responding between 20% and 80% was considered as evidence for partial substitution. The dose–response curves were subject to two-factor (dose, isomer) analyses of variance. The results of time course tests were also subject to two-factor (time interval, test drug) ANOVAs. ED_{50}s were calculated from dose–response
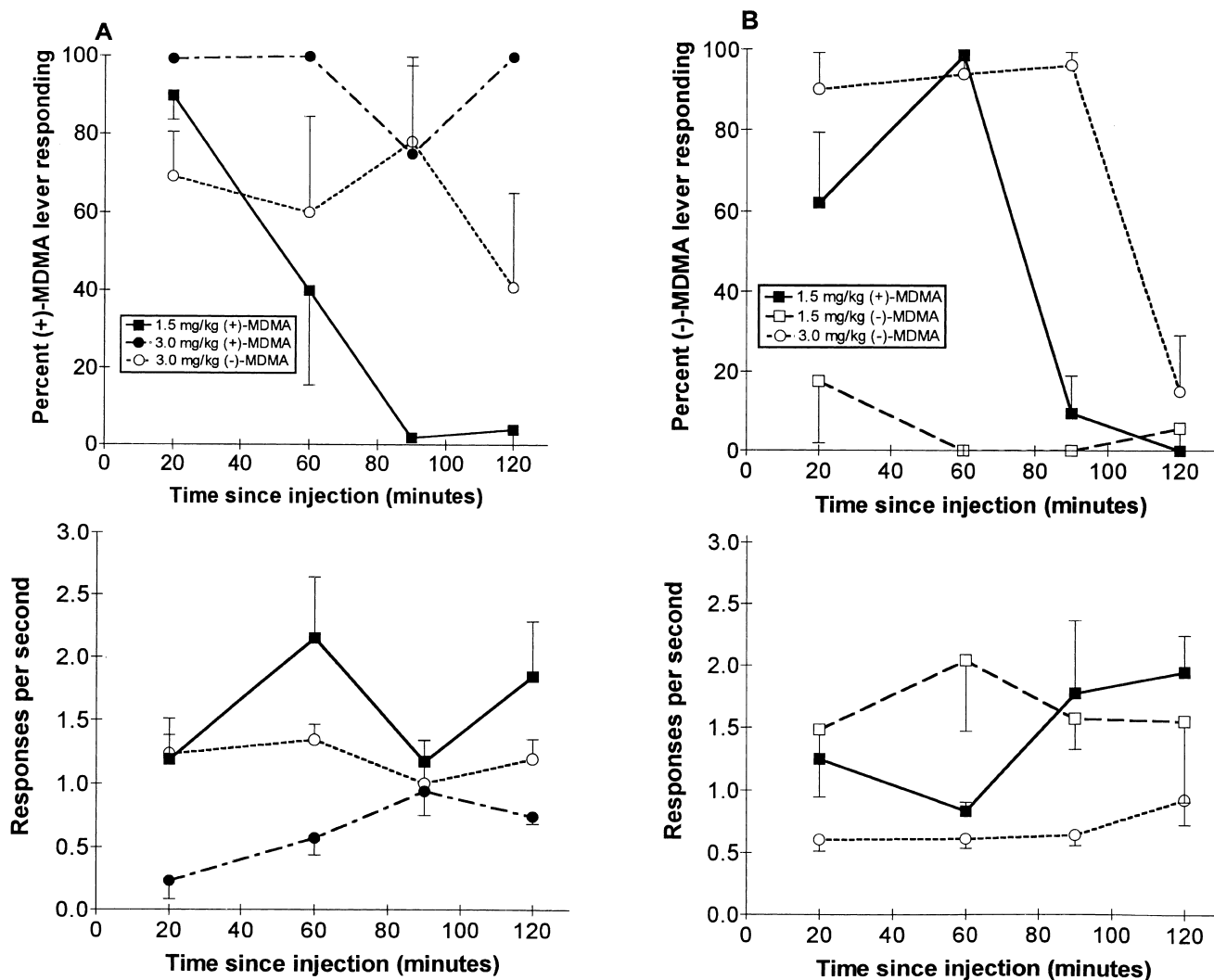
FIG. 3. A: Substitution tests with (+)-MDMA (1.5 and 3.0 mg/kg) and (−)-MDMA (3.0 mg/kg) at 20, 60, 90 and 120 min after injection in animals trained to discriminate (+)-MDMA. Except where noted in text, each data point represents the mean of five animals. B: Substitution tests with (−)-MDMA (1.5 and 3.0 mg/kg) and (+)-MDMA (1.5 mg/kg) at 20, 60, 90 and 120 min after injection in animals trained to discriminate (−)-MDMA. Except where noted in text, each data point represents the mean of seven animals. Percentage total responses on the training drug lever is shown above and response rate is shown below.

functions with each isomer. The statistical analyses and ED$_{50}$ calculations were performed with the statistical software GraphPad Prism (GraphPad, Inc.).

RESULTS

Six of the eight subjects administered training sessions with (+)-MDMA (1.5 mg/kg) attained the discrimination criterion within an average of 48 training sessions (SD = 10.18; range = 37–66). Seven of the eight subjects administered training sessions with (−)-MDMA attained the discrimination criterion within an average of 50 training sessions (SD = 12.06; range = 37–66). One rat in each training group died early in the study before the discrimination criterion could be met. An additional rat in the (+)-MDMA training group was killed later in the study due to an illness, although this rat had not attained the discrimination criterion after 138 training sessions. Thus,

five rats trained to discriminate (+)-MDMA and seven rats trained to discriminate (−)-MDMA were administered substitution tests with each isomer.

Figure 2 illustrates the dose–response curves and indicates the ED$_{50}$s for both isomers of MDMA in animals trained to discriminate either (+)-MDMA (Fig. 2A) or (−)-MDMA (Fig. 2B) from saline. The ED$_{50}$ for (+)-MDMA was lower than that of (−)-MDMA in rats trained to discriminate either isomer. The 0.75 mg/kg dose of (+)-MDMA nearly substituted (78%) for the training dose of this isomer, although twice the training dose was required to show stimulus generalization in (−)-MDMA-trained rats. Rats trained to discriminate (+)-MDMA did not generalize completely to (−)-MDMA. In fact, the training dose of each isomer produced equivalent amounts of drug-appropriate responding (62%) in rats trained to discriminate the opposite isomer. A dose of 4.0 mg/ kg (−)-MDMA was tested in four (+)-MDMA trained rats

and was highly disruptive; only one animal completed 20 responses, but these were all on the drug lever (data not shown). Interestingly, lower doses of (−)-MDMA (0.75 and 1.5 mg/kg) produced more drug-appropriate responding in the animals trained to discriminate (+)-MDMA than in animals trained to discriminate (−)-MDMA. The results of statistical analyses of these data are summarized in Table 1. In the animals trained to discriminate (+)-MDMA, there was only a significant main effect of dose on percentage of drug-appropriate responding and on response rate. In the animals trained to discriminate (−)-MDMA, the main effects of dose and test drug and the interaction between these factors on percentage of drug-lever responding were all significant. Response rate was also significantly affected by dose in these subjects.

Figure 3 illustrates the changes in the amount of discriminative stimulus control maintained by (+)-MDMA and (−)-MDMA over time. In the rats trained to discriminate (+)-MDMA (Fig. 3A), only partial generalization occurred when the training dose was administered 60 min prior to test sessions. The mean of 40% represents two subjects that emitted all of their responses on the drug lever and three subjects that responded entirely on the saline lever. At the 90- and 120-min intervals, fewer than 5% of the responses were made on the drug-appropriate lever. However, when twice the training dose was administered, discriminative stimulus control was maintained 2 h after injection. Only four subjects were tested with 3.0 mg/kg (+)-MDMA 90 min after injection; three subjects emitted 100% of their responses on the drug-appropriate lever and one emitted 100% of its responses on the saline lever.

In the rats trained to discriminate (−)-MDMA (Fig. 3B), the training dose maintained discriminative stimulus control 60 and 90 min after injection but not 120 min after injection. As seen in the previous dose–response test, 1.5 mg/kg (−)-MDMA did not substitute for the training dose of this isomer, and the absence of stimulus control by this dose was demonstrated at several time intervals. The training dose of (−)-MDMA (3.0 mg/kg) was also tested for stimulus generalization in the (+)-MDMA-trained animals at different time intervals following injection. As noted in the results of the dose–response tests, only partial substitution was observed with this dose. Surprisingly, the 1.5-mg/kg dose of (+)-MDMA produced complete stimulus generalization in rats trained to discriminate (−)-MDMA when administered 60 min prior to test sessions.

The statistical analyses on the results of time course tests are also illustrated in Table 1. In both training groups, two-factor ANOVAs on percentage of drug-lever responding revealed significant main effects of test drug and time and a significant interaction between these factors. Only the main effect of test drug was significant on response rate in both training groups.

DISCUSSION

The ability of MDMA to establish and maintain discriminative stimulus control over lever-pressing behavior of rats was first demonstrated by Glennon et al. in 1986 (13). Schechter (25) subsequently demonstrated that both of the MDMA enantiomers substitute for the racemate. Several reports followed that described attempts to characterize the discriminative stimulus effects of the MDMA isomers by testing these agents in animals trained to discriminate other drugs such as psychostimulants (4,14,22) or hallucinogens (5). A recent report provided documentation of the first attempt to characterize the discriminative stimulus effects of the MDMA isomers in animals trained to discriminate the individual iso-

mers from saline (2). The present study attempted to replicate some of those findings in a different laboratory, with slightly modified training procedures, and extend those findings by investigating the time course of the discriminative stimulus effects of each enantiomer of MDMA.

The results of experiment 1 support previous findings (2) that 1.25 mg/kg (+)-MDMA is capable of establishing discriminative stimulus control in rats when administered 20 min prior to training sessions. However, the present results indicate that at this low dose (+)-MDMA is not capable of establishing discriminative stimulus control when administered 90 min before the onset of training sessions. In contrast, (−)-MDMA (3.5 mg/kg), at a dose previously shown to substitute for (+)-MDMA (1.25 mg/kg) (2), established discriminative stimulus control when administered either 20 min or 90 min prior to the onset of training sessions. The second experiment was conducted to investigate further the possible differences in the time course of the discriminative stimulus effects of each isomer, although slightly different training doses were used. The results indicate that (−)-MDMA (3.0 mg/kg) maintains discriminative stimulus control at longer postinjection intervals than (+)-MDMA (1.5 mg/kg). When 3.0 mg/kg (−)-MDMA was administered 60 or 90 min prior to test sessions, complete substitution was observed in animals trained to discriminate this isomer. In contrast, rats trained to discriminate (+)-MDMA did not generalize to this dose of (+)-MDMA when administered 60 min or 90 min prior to test sessions. Schechter (26) reported that racemic MDMA (2.5 mg/kg) establishes discriminative stimulus control in rats when administered 105 min before training sessions. When considered with the present results, the discrimination of MDMA at a longer injection interval may be attributed primarily to the effects of the (−)-isomer. However, dose must also be considered. Schechter (26) reported that 1.5 mg/kg MDMA was not discriminated 105 min after injection. Because the second experiment in the present study showed that 3.0 mg/kg (+)-MDMA is discriminated 120 min after injection, it would be premature to conclude that the discrimination of MDMA (2.5 mg/kg) at 105 min is due primarily to the effects of the (−)-isomer. Nevertheless, the present results suggest that, at doses that produce similar amounts of cross generalization, (−)-MDMA appears to be capable of maintaining discriminative stimulus control at longer postinjection intervals than (+)-MDMA.

The results of the second experiment also confirmed previous findings (2) that (+)-MDMA is more potent than (−)-MDMA. This potency is evident by the lower ED_{50} observed with (+)-MDMA in rats trained to discriminate either isomer. Although previous investigations have demonstrated cross substitution between 1.25 mg/kg (+)-MDMA and 3.5 mg/kg (−)-MDMA (2), only partial substitution was observed between the training doses of the two isomers in the present study. A higher dose of (+)-MDMA (3.0 mg/kg) did substitute completely for (−)-MDMA, but a higher dose of (−)-MDMA (4.0 mg/kg) severely disrupted responding in (+)-MDMA-trained rats. The difference in the amount of cross generalization observed between the two isomers in experiment 2 and that found in a previous study (2) could be accounted for by slightly different training doses or other differences in the training procedures. The ED_{50} determined for (+)-MDMA in animals trained to discriminate this isomer was very similar in experiments 1 and 2 of the present study and in the previous study (2). However, the ED_{50} determined for (−)-MDMA in animals trained to discriminate this isomer was slightly higher in the second experiment (2.71 mg/kg) than in either the first experiment (1.02 mg/kg in the 20-min

group, 1.32 mg/kg in the 90-min group) or in the previous study (2) (1.85 mg/kg). Also, the present finding that (−)-MDMA was approximately three times more potent in animals trained to discriminate (+)-MDMA than in those trained to discriminate (−)-MDMA is of interest. This finding may be attributed to the fact that the (+)-MDMA-trained animals were trained to detect a lower dose of a drug with similar (but not necessarily the same) stimulus properties as (−)-MDMA.

The results of experiment 1 confirmed previous findings that (+)-amphetamine does not substitute for either isomer of MDMA (2) and supports reports from other investigators (11,26) that rats trained to discriminate racemic MDMA do not generalize to (+)-amphetamine. Despite the difficulty in interpreting partial substitution it is of interest that (+)-amphetamine produced a greater amount of drug-appropriate responding in animals trained to discriminate (−)-MDMA 90 min after injection than in animals trained to discriminate this isomer 20 min after injection. Higher doses of (+)-amphetamine were not assessed in the present study because previous investigations have shown that (+)-amphetamine doses higher than 1.0 mg/kg severely disrupt responding in animals trained to discriminate MDMA (22) or its isomers (2). Moreover, response rate was reduced drastically by 1.5 mg/kg (+)-amphetamine in the present study.

Although (+)-amphetamine did not substitute for either MDMA isomer, the present findings that (+)-amphetamine produced a greater amount of drug-appropriate responding in the (+)-MDMA at 20 min-trained animals than in the (−)-MDMA at 20 min trained animals is of interest and lends some support to Glennon et al.'s (14) findings that (+)-MDMA is more similar than (−)-MDMA to (+)-amphetamine. However, Oberlender and Nichols (22) found that neither MDMA isomer is substituted for (+)-amphetamine. Although both of these studies reported using a training dose of 1.0 mg/kg (+)-amphetamine, the presession injection interval was 15 min in the study by Glennon et al. (14) and 30 min in the study by Oberlender and Nichols (22). Moreover, there were several other methodological differences between these studies. For example, Glennon et al. employed a VI 15-s schedule of reinforcement during discrimination training sessions, whereas Oberlender and Nichols employed a FR 50 schedule of reinforcement during discrimination training sessions. Also, subjects that made fewer than five responses in 2.5-min test sessions were considered disrupted in the study by Glennon et al., whereas subjects that made fewer than 50 responses in 5-min test sessions were considered disrupted and their data eliminated from the analyses in the study by Oberlender and Nichols. The training and testing procedures employed in the present study differed from those described in both of these previous reports. Without a systematic experimental analysis of different methodological parameters of drug-discrimination research, it is difficult to make direct comparisons between the present results and those of other studies.

The observation that (+)-MDMA appears to produce a stronger (+)-amphetamine-like component than (−)-MDMA coincides with neurochemical evidence that (+)-MDMA is a more potent DA releaser than (−)-MDMA (19). The fact that at least partial antagonism of the (+)-MDMA-20-min cue was observed with Sch 39166, whereas this selective D1 antagonist had little effect on drug-appropriate responding in animals trained to discriminate (−)-MDMA at 20 min, is also consistent with the neurochemical differences between the MDMA isomers. In addition, partial antagonism of the (−)-MDMA-90-min cue was produced by Sch 39166. It is tempting to interpret these findings as evidence for greater dopaminergic activity at longer postinjection intervals. However, this conclusion should be considered with caution because Sch 39166 produced only partial antagonism, and these effects were not dose-dependent. Furthermore, statistical analyses revealed no reliable main effect of training group or dose × training group interaction. Also, the observation that the D2 dopamine antagonist haloperidol reduced (−)-MDMA-appropriate responding in both the 20-min and the 90-min training groups to a similar extent contradicts this conclusion. Schechter (26) demonstrated that haloperidol reduced MDMA discrimination to a greater degree in animals trained to discriminate racemic MDMA 105 min after injection than in animals trained to discriminate this drug 20 min after injection. The present results in rats trained to discriminate (−)-MDMA fail to support those findings.

The present results do support previous findings that fenfluramine substitutes for both isomers of MDMA and confirm the notion that 5-HT release is a more critical component than DA release in mediating the discriminative stimulus effects of both MDMA isomers (2). Unfortunately, only partial substitution was observed in the (−)-MDMA-20-min training group when tested with 4 mg/kg fenfluramine (group mean = 75%). In a previous study (2), this dose of fenfluramine substituted completely for (−)-MDMA (3.5 mg/kg). However, five of eight animals in the (−)-MDMA-20-min training group in the present study made 100% of their responses on the drug lever, and the ED_{50} was actually lower than that found in the previous study (1.42 vs. 1.91 mg/kg). This slight discrepancy between results could be explained by some minor differences in training and testing procedures employed in the two studies. For example, in the first study (2), animals were not required to make 20 consecutive responses to receive reinforcement during training sessions. This minor difference in training history could account for different results during substitution tests. In addition, substitution tests were administered more frequently in the present study than in the previous study. Because this high dose of fenfluramine decreases serotonin levels with frequent administration (16), the lack of substitution with 4 mg/kg fenfluramine in three animals in the present study might be due to the effects of too frequent testing. A recent report provided evidence that repeated (+)-fenfluramine administration (4.0 mg/kg twice a day for 4 days) alters MDMA discrimination 2 weeks later (3). Whether the pattern of fenfluramine administration in substitution tests in the present study produced sufficient serotonin reduction to alter MDMA discrimination is undetermined at this time.

The importance of serotonergic mediation of the discriminative stimulus effects of racemic MDMA was previously documented by findings that norfenfluramine and TFMPP substitute for MDMA (26). Furthermore, investigations of 5-HT antagonists in combination with MDMA have revealed that 5-HT$_2$ receptors (26) and 5-HT$_3$ receptors (15) may be important in mediating the discriminative stimulus effects of this compound. Despite differences in training and dosing procedures, both Schechter (26) and Glennon et al. (15) showed that the 5-HT$_2$ antagonist pirenpirone reduces the percentage of MDMA-appropriate responding to about 40%. However, the 5-HT$_2$ antagonist ketanserin had little effect on MDMA-appropriate responding (15). In contrast, the 5-HT$_3$ antagonists zacopride and LY 278584 reduced MDMA-appropriate responding to about 20% (15). Glennon et al. (15) also reported that the 5-HT$_{1A}$ antagonist NAN-190 reduced

MDMA-appropriate responding to about 60%, although it severely disrupted responding at 1.0 mg/kg. The lack of antagonism with the 5-HT$_{1A}$ antagonist WAY 100,135 in the present study is consistent with previous findings that NAN-190 does not block the discriminative stimulus effects of racemic MDMA (15). However, conclusions regarding the importance of 5-HT$_{1A}$ receptors in mediating the discriminative stimulus effects of the MDMA isomers should be considered preliminary until higher doses of WAY 100,135 are examined. Due to a limited drug supply, this was not possible in the present study.

In summary, the present study extends previous findings that the discriminative stimulus effects of the MDMA isomers may differ is some subtle but perhaps important ways. First, (+)-MDMA is capable of establishing discriminative stimulus control at lower doses than (−)-MDMA. In addition, at doses that produce approximately equivalent amounts of drug-appropriate responding, (−)-MDMA appears to be capable of maintaining discriminative stimulus control for longer periods following injection. The higher potency of (+)-MDMA in its behavioral actions and the fact that (+)-MDMA is a more potent DA releaser suggest that DA release is an important mechanism underlying the behavioral effects of MDMA. However, the lack of consistent antagonism of either isomer of MDMA with DA antagonists suggests that DA mechanisms play a minor role in the ability of these drugs to establish and maintain discriminative stimulus control. Of the antagonists tested in the present study, only pirenpirone produced orderly, dose-dependent decreases in drug-appropriate responding. However, based on the statistical analysis of the data, no reliable effects were observed as a function of training condition. As previous investigations have noted (15,26), MDMA produces complex discriminative stimulus effects that involve both serotonergic and dopaminergic mechanisms, and 5-HT$_2$ receptor activation is more important than dopamine D1 or D2 receptor activation. The suggestion that dopaminergic mechanisms may be more important at longer presession injection intervals (26) is not well supported by the present data. Further investigations are required to determine the importance of DA release in mediating the cue properties of (+)-MDMA at different time periods following injection, and it is recommended that higher doses of (+)-MDMA be examined in such studies.

REFERENCES

1. Anderson, G. M.; Braun, G.; Braun, U.; Nichols, D. E.; Shulgin, A. T.: Absolute configuration and psychotomimetic activity. NIDA Res. Monogr. Ser. 22:8–15; 1978.
2. Baker, L. E.; Broadbent, J.; Michael, E. K.; Matthews, C. A.; Metosh, R. B.; West, W. B.; Appel, J. B.: Assessment of the discriminative stimulus effects of the optical isomers of ecstasy (3,4-methylenedioxymethamphetamine; MDMA). Behav. Pharmacol. 6:263–275; 1995.
3. Baker, L. E.; Makhay, M. M.: Effects of (+)-fenfluramine on 3,4-methylenedioxymethamphetamine (MDMA) discrimination in rats. Pharmacol. Biochem. Behav. 53:455–461; 1996.
4. Broadbent, J.; Michael, E. K.; Appel, J. B.: Generalization of cocaine to the isomers of 3,4-methylenedioxyamphetmine and 3,4-methylenedioxymethamphetamine: effects of training dose. Drug Dev. Res. 16:443–450; 1989.
5. Callahan, P. M.; Appel, J. B.: Differences in the stimulus properties of 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine in animals trained to discriminate hallucinogens from saline. J. Pharmacol. Exp. Ther. 246:866–870; 1988.
6. Chait, L. D.; Uhlenhuth, E. H.; Johanson, C. E.: The discriminative stimulus and subjective effects of d-amphetamine, phenmetrazine and fenfluramine in humans. Psychopharmacologia 89:301–306; 1986.
7. Climko, R. P.; Roehrich, H.; Sweeney, D. R.; Al-Razi, J.: Ecstasy: a review of MDMA and MDA. Int. J. Psychiatr. Med. 16:359–372; 1987.
8. Evans, S. M.; Johanson, C. E.: Discriminative stimulus properties of (+/−)-3,4-methylenedioxymethamphetamine and (+/−)-3,4-methylenedioxyamphetamine in pigeons. Drug Alcohol Depend. 18:159–164; 1986.
9. Evans, S. M.; Zancy, J. P.; Johanson, C. E.: Three-choice discrimination among (+)-amphetamine, fenfluramine and saline in pigeons. Pharmacol. Biochem. Behav. 35:971–980; 1990.
10. Extance, K.; Goudie, A. J.: Inter-animal olfactory cues in operant drug discrimination procedures in rat. Psychopharmacologia 91:67–73; 1981.
11. Glennon, R. A.; Misenheimer, B. R.: Stimulus effects of N-monoethyl-1-(3,4-methylenedioxy phenyl)-2-aminopropane (MDE) and N-hydroxy-1-(3,4-methylenedioxyphenyl)-2-aminopropane (N-OH

MDA) in rats trained to discriminate MDMA from saline. Pharmacol. Biochem. Behav. 33:909–912; 1989.
12. Glennon, R. A.; Young, R.; Rosecrans, J. A.; Anderson, G. M.: Discriminative stimulus properties of MDA analogs. Biol. Psychol. 17:807–814; 1982.
13. Glennon, R. A.; Titelerm, M.; Lyon, R. A.; Yousif, M.: MDMA ("Ecstasy"): drug discrimination and brain binding properties. Soc. Neurosci. Abstr. 12:919; 1986.
14. Glennon, R. A.; Yousif, M.; Patrick, G.: Stimulus properties of 1-(3,4-methylenedioxyphenyl)-2-aminopropane (MDA) analogs. Pharmacol. Biochem. Behav. 29:443–449; 1988.
15. Glennon, R. A.; Higgs, R.; Young, R.; Issa, H.: Further studies on N-methyl-1(3,4-methylenedioxyphenyl)-2-aminopropane as a discriminative stimulus: antagonisim by 5-hydroxytryptamine3 antagonists. Pharmacol. Biochem. Behav. 43:1099–1106; 1992.
16. Harvey, J. A.; McMaster, S. E.: Fenfluramine: evidence for a neurotoxic action on a long-term depletion of serotonin. Psychopharmacol. Commun. 1:217–228; 1975.
17. Johnson, M. P.; Hoffman, A. J.; Nichols, D. E.: Effects of the enantiomers of MDA, MDMA and related analogues on 3H-serotonin and 3H-dopamine release from superfused rat brain slices. Eur. J. Pharmacol. 132:269–276; 1986.
18. Lyon, R. A.; Glennon, R. A.; Titeler, M.: 3,4-Methylenedioxymethamphetamine (MDMA): stereoselective interactions at 5-HT1 and 5-HT2 receptors. Psychopharmacologia 88:525–526; 1986.
19. McKenna, D. J.; Guan, X. M.; Shulgin A. T.: 3,4-methylenedioxyamphetamine (MDA) analogues exhibit differential effects on synaptosomal release of 3H-dopamine and 3H-5-hydroxytryptamine. Pharmacol. Biochem. Behav. 38:505–512; 1990.
20. Nichols, D. E.: Differences between the mechanism of action of MDMA, MBDB and the classic hallucinogens. Identification of a new therapeutic class: entactogens. J. Psychoact. Drugs. 18:305–313; 1986.
21. Nichols, D. E.; Hoffman, A. J.; Oberlender, R. A.; Jacob, P. III; Shulgin, A. T.: Derivatives of 1-(1,3-benzodioxol-5-yl)-2-butanamine: representatives of a novel therapeutic class. J. Med. Chem. 29:2009–2015; 1987.
22. Oberlender, R.; Nichols, D. E.: Drug discrimination studies with MDMA and amphetamine. Psychopharmacologia 95:71–76; 1988.

23. Peroutka, S. J.; Newman, H.; Harris, H.: Subjective effects of 3,4-methylenedioxymethamphetamine in recreational users. Neuropsychopharmacology 1:273–277; 1988.
24. Schechter, M. D.: Discriminative profile of MDMA. Pharmacol. Biochem. Behav. 24:1533–1537; 1986.
25. Schechter, M. D.: MDMA as a discriminative stimulus: isomeric comparisons. Pharmacol. Biochem. Behav. 27:41–44; 1987.
26. Schechter, M. D.: Serotonergic-dopaminergic mediation of 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy"). Pharmacol. Biochem. Behav. 31:817–824; 1989.
27. Schechter, M. D.; Cooke, P. G.: Dopaminergic mediation of the interoceptive cue produced by *d*-amphetamine in the rat. Psychopharmacologia 42:185–193; 1975.
28. Schmidt, C. J.; Fadayel, G. M.; Sullivan, C. K.; Taylor, V. L.: 5-HT2 receptors exert a state-dependent regulation of dopaminergic function: studies with MDL 100,907 and the amphetamine analogue, 3,4-methylenedioxymethamphetamine. Eur. J. Pharmacol. 223:65–74; 1992.
29. Shulgin, A. T.; Nichols, D. E.: Characterization of three new psychotomimetics. In: Stillman, R. C.; Willette, R. E., eds. The psychopharmacology of hallucinogens. New York: Pergamon Press; 1978:74–83.