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Effects of (+)-Fenfluramine on 3,4-Methylenedioxymethamphetamine (MDMA) Discrimination in Rats

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BAKER, L. E. AND M. M. MAKHAY. *Effects of (+)-fenfluramine on 3,4-methylenedioxymethamphetamine (MDMA) discrimination in rats.* PHARMACOL BIOCHEM BEHAV **53**(2) 455–461, 1996.—This study examined the effects of a presumed neurotoxic dose regimen of (+)-fenfluramine on the discrimination of MDMA and (+)-amphetamine in male Sprague–Dawley rats trained to discriminate 1.5 mg/kg MDMA from saline in a two-choice operant task. Substitution tests were conducted with saline, several doses of MDMA (0.19–1.5 mg/kg), and (+)-amphetamine (0.125–1.0 mg/kg) prior to and again following the administration of (+)-fenfluramine (4.0 mg/kg twice a day for 4 days; $n = 11$) or a similar pattern of saline injections ($n = 10$). During pretreatment substitution tests, lower doses of MDMA elicited drug-appropriate responding in a dose-dependent manner, although none of these doses substituted for the training dose. Likewise, no dose of (+)-amphetamine substituted for the training drug during pretreatment substitution tests. The discrimination of MDMA was disrupted in some animals following (+)-fenfluramine treatment, but with subsequent training, discrimination criteria were met. In posttreatment substitution tests, the lowest dose of MDMA produced significantly higher drug-appropriate responding in (+)-fenfluramine treated animals but not in saline-treated animals. The amount of drug-appropriate responding during posttreatment substitution tests with (+)-amphetamine varied little from pretreatment substitution tests in saline-treated animals, but was greater at all doses in (+)-fenfluramine-treated animals; the highest dose of (+)-amphetamine substituted for MDMA subsequent to (+)-fenfluramine treatment. These results support previous findings that the long-lasting serotonergic effects of fenfluramine may have functional consequences that can be detected using a drug discrimination procedure. Specifically, serotonin depletion may unmask or strengthen the stimulant-like effects of MDMA.

Drug discrimination Rats MDMA (+)-Fenfluramine (+)-Amphetamine Serotonin Dopamine

3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA, Ecstasy) is a ring-substituted phenylisopropylamine, structurally similar to the psychomotor stimulant amphetamine and the hallucinogen mescaline. Investigations on the discriminative stimulus effects of MDMA indicate that the cue properties of this compound are similar, yet perhaps more complex than either stimulants or hallucinogens (9,33). The subjective effects of this compound in humans have been described as intensified affect, increased self-esteem, and enhanced communication and intimacy without the sensory distortion commonly associated with classical hallucinogens (5,31). Because the subjective effects of this compound appear to be distinct from stimulants and hallucinogens, some researchers have suggested that MDMA deserves a unique classification [e.g., entactogens; (28,29)].

It is well documented that MDMA facilitates the presynaptic release of serotonin (5-HT) and dopamine (DA) in vitro and in vivo (23,38). Drug discrimination experiments have indicated that the stimulus effects of MDMA may be mediated to a greater extent by 5-HT than by DA because the nonselective 5-HT agonists norfenfluramine and N-(3-trifluoromethylphenyl)piperazine (TFMPP) substituted reliably for MDMA, whereas the dopaminergic agonists, (+)-amphetamine and (−)-cathinone did not (33). Also, both 5-HT_2 and 5-HT_3 antagonists have been reported to significantly decrease MDMA discrimination, whereas the DA antagonist haloperidol has been reported to have little effect on the MDMA cue (15,33). Schechter (32) also demonstrated that in animals trained to discriminate fenfluramine from saline, drug-appropriate responding generalized to the effects of MDMA. In addition,

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recent investigations demonstrated that fenfluramine and another 5-HT releaser, *p*-chloroamphetamine, substituted reliably and potently for both stereoisomers of MDMA (1).

Although the serotonergic component of MDMA's stimulus effects is apparent, the extent to which dopaminergic mechanisms play a role in these (and other behavioral) effects of MDMA requires further investigation. Drug discrimination experiments on the similarities between MDMA and (+)-amphetamine (a potent DA releaser) have revealed inconsistent results. Although several investigators have reported that MDMA mimics the stimulus effects of (+)-amphetamine (7,13,25), this substitution appears to be asymmetrical because (+)-amphetamine does not substitute for MDMA (33). However, a report by Oberlander and Nichols (30) revealed the opposite results (i.e., MDMA did not substitute for (+)-amphetamine, but (+)-amphetamine did substitute for MDMA).

There have also been inconsistent reports regarding the similarities between (+)-amphetamine and the MDMA stereoisomers. Glennon et al. (14) demonstrated that (+) MDMA but not (−) MDMA substituted for (+)-amphetamine, while Oberlander and Nichols (30) found neither isomer to substitute for (+)-amphetamine. Other investigators have shown that in animals trained to discriminate the individual stereoisomers of MDMA from saline, (+)-amphetamine did not substitute for either compound (1).

Fenfluramine is also a derivative of amphetamine; however, despite their structural similarities and common therapeutic effects as anorectic agents, amphetamine and fenfluramine produce markedly different behavioral effects. Amphetamine increases locomotor activity in rodents (6), while fenfluramine has sedative effects (41); humans report stimulant-like subjective effects (4) under the influence of amphetamine, while fenfluramine possesses a nonstimulant subjective profile (4,17,19). Furthermore, amphetamine is self-administered by rhesus monkeys (2) and humans (22), whereas fenfluramine is not (22,40). These behavioral differences are more than likely related to neuropharmacological differences; amphetamine's effects are primarily mediated by DA release (16), while the effects of fenfluramine are mediated by 5-HT release (21).

Although some drug discrimination investigations have indicated some overlap between the cue properties of fenfluramine and amphetamine (4,8,18), the stimulus effects of these compounds were recently shown to be distinct using a three-choice discrimination procedure in pigeons (9). Of particular interest were the findings that MDMA produced both amphetamine- and fenfluramine-appropriate responding, further indication that MDMA has multiple stimulus effects (9).

Given the unique profile of MDMA's subjective effects in humans, it is not surprising that this compound exhibits multiple stimulus effects in other species. That cross-substitution occurs between fenfluramine and MDMA (32,33) but not between (+)-amphetamine and MDMA (7,13,25,33) may indicate that 5-HT release is a primary component of MDMA's complex stimulus properties while DA release plays a minor role. One way to assess the relative importance of 5-HT release in mediating the cue properties of MDMA is to investigate the impact of 5-HT depletion on MDMA discrimination. Pretreatment with *p*-chlorophenylalanine, a reversible 5-HT depletor, was shown to decrease discriminative performance in animals trained to detect the cue properties of the serotonin releasing drugs MDMA, N-ethyl-3,4-methylenedioxyamphetamine (MDE) and fenfluramine (36) but did not interfere with the discrimination of dopaminergically mediated compounds. In another study, the same investigator demonstrated that a neurotoxic regimen of MDMA decreased the discriminability of lower doses of MDMA (35). In contrast, administration of (±)-fenfluramine at doses known to produce serotonin depletion significantly enhanced the discrimination of subthreshold doses of (±)-fenfluramine (34). Other investigators have demonstrated that repeated MDMA administration produced serotonin depletion and enhanced the psychomotor stimulant effects of acute MDMA administration on DRL schedule-controlled responding (27). Li et al. (27) suggested that 5-HT normally exerts an inhibitory action on the psychomotor stimulant effects of MDMA and that depletion of 5-HT allows MDMA to exert a stronger stimulant-like effect.

Like MDMA, fenfluramine has long-lasting, possibly neurotoxic effects on brain serotonin systems. The prolonged depletion of 5-HT and 5-HIAA as a consequence of high doses of (±)-fenfluramine (20) has been compared to the neurotoxicity produced by (±) MDMA (3). Other investigators have determined that these effects of fenfluramine are stereoselective; (+)-fenfluramine decreased 5-HT and 5-HIAA in the rat cortex and hippocampus, while (−)-fenfluramine was relatively ineffective (24). The present study assessed the effects of a presumed neurotoxic dose regimen of (+)-fenfluramine on the discriminative stimulus properties of MDMA to determine if such treatment would enhance the amphetamine-like effects of MDMA. Substitution of (+)-amphetamine for MDMA was examined before (+)-fenfluramine treatment and reassessed following subsequent MDMA discrimination training.

METHOD

Subjects

Thirty-two male Sprague–Dawley rats (Harlan, Indianapolis, IN), aged 6 to 8 months and weighing 350–400 g at the beginning of the study, were used. Subjects had been previously exposed to operant training on a single lever in an undergraduate learning lab. All subjects were drug naive prior to the onset of the present study. Animals were individually housed in wire mesh cages, in a colony maintained on a 12 L : 12 D lights (0700 to 1900) cycle and at constant temperatures (20–22°C). Water was provided ad lib, 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 using MED-PC instrumentation and software (MED Associates Inc., St. Albans, VT, version 2.0) to control experimental events and data collection.

Drugs

(±) MDMA, (+)-amphetamine, and (+)-fenfluramine were obtained from the National Institute on Drug Abuse (Rockville, MD). All drug doses are expressed as the salt. Drugs were dissolved in 0.85% physiological saline and administered intraperitoneally.

Behavioral Procedures

Discrimination training. Subjects were trained to discriminate (±) MDMA (1.5 mg/kg) from saline in a two-choice operant task under a fixed-ratio 20 (FR20) schedule. For half the animals, responding on the right lever was reinforced with sweetened condensed milk (diluted 1 : 1 in tap water) following 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), both levers were wiped with isopropyl alcohol before each session. MDMA or saline injections were administered intraperitoneally (IP), 20 min prior to 30 min training sessions that were conducted 6 days per week (Monday through Saturday). The first two training sessions were conducted under saline-appropriate conditions; thereafter, MDMA and saline conditions were presented in a semirandom order with the restriction that neither condition be presented for more than two consecutive sessions. Training under each condition began under an FR 1 schedule and when responding was stable, the number of consecutive correct responses required for reinforcement was gradually increased from 1 to 20 (FR 20). Responding on the incorrect lever reset the counter. Substitution tests commenced when animals maintained response choice accuracies of at least 85% prior to delivery of the first reinforcer for 10 consecutive sessions.

Stimulus substitution testing. Substitution tests were conducted with saline, (±) MDMA (0.1875–1.5 mg/kg), and (+)-amphetamine (0.125–1.0 mg/kg) prior to and again following a presumably neurotoxic regimen of (+)-fenfluramine administration. During test sessions, animals were injected with the test solution (20 min preinjection interval) and placed in the chambers for 30 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 upon completion of the test. Test sessions were conducted once or twice per week on different days of the week, providing the animals maintained discrimination criterion during training sessions. Half of the animals were administered all test sessions on days after a drug training session and half were administered all test sessions on days after a saline-training session. For the pretreatment substitution tests, all doses of MDMA were tested first, followed by substitution tests with (+)-amphetamine. For the posttreatment substitution tests, test doses of MDMA and (+)-amphetamine were administered in an increasing, alternating order (MDMA dose 1, AMPH dose 1, MDMA dose 2, AMPH dose 2, etc.).

Fenfluramine administration. Animals that completed the first set of substitution tests (n = 19) were randomly assigned to two treatment groups. One group (n = 10) was administered (+)-fenfluramine (4.0 mg/kg twice a day for 4 days) and the other group (n = 9) was administered the same regimen of saline injections. Two animals that had completed all but two of the pretreatment substitution tests were also administered the above regimen of (+)-fenfluramine (n = 1) or saline (n = 1) injections. Animals were allowed to recover for 10 days before resuming training. A minimum of three training sessions (two saline training sessions followed by one MDMA training session) were given before the administration of posttreatment substitution tests with MDMA and (+)-amphetamine were begun. Each animal was required to meet the discrimination criterion of 85% or better (prior to delivery of first reinforcer) under each training condition before resuming testing.

Data Analyses

Substitution data are presented as the percent of total responses made on the drug-appropriate lever during test sessions. Response rate is indicated as the number of responses made (on either lever) per second during test sessions. The means are reported for each treatment group at each dose level tested during pretreatment and posttreatment tests. A particular dose was considered to substitute for the training drug if the mean percent drug-appropriate responding was 80% or greater. The SAS General Linear Model procedure was used to conduct a two-factor (factor 1 = dose, factor 2 = time, or pretreatment vs. posttreatment) repeated measures analysis of variance on percent drug-appropriate responding and on response rate for each drug tested. For control tests only (saline, MDMA 1.5 mg/kg), group means for a particular test were substituted for missing data for statistical analyses.

RESULTS

The number of training sessions required to achieve the discrimination criterion (10 consecutive days above 85% on the first FR) ranged from 22 to 80, with a mean of 44 sessions. When posttreatment discrimination training resumed, a minimum of three training sessions were given prior to administering substitution tests. However, several animals required more sessions to achieve the performance criterion of 85% under both training conditions. The number of training sessions required to resume testing in the (+)-fenfluramine-treated animals ranged from 4 to 11 sessions; the number required for the saline-treated animals ranged from three to nine sessions.

Table 1 summarizes the mean percent drug-appropriate responding up to completion of the first FR (prior to any reinforcement) during the first saline training session and the first MDMA training session following (+)-fenfluramine or saline treatment. Percent drug-appropriate responding by the (+)-fenfluramine-treated animals during the first posttreatment training session with MDMA was clearly below the 85% criterion. In fact, only 4 of the 11 animals that received (+)-fenfluramine treatment made greater than 95% of their responses on the drug-appropriate lever on the first FR; the other seven animals achieved less than 50% on the first FR during the first posttreatment MDMA training session. In contrast, 8 of the 10 saline-treated animals responded more than 95% on the drug-appropriate lever for the first FR; one made 80% and one made less than 50%.

Table 2 summarizes the results of substitution tests with MDMA and (+)-amphetamine in both saline and (+)-fenfluramine-treated animals before and after treatment. The number of animals tested at each dose during the posttreat-

TABLE 1

DISCRIMINATION PERFORMANCE DURING FIRST MDMA SESSION AND FIRST SALINE SESSION FOLLOWING TREATMENT

		Percent Drug-Appropriate Responding Upon Completion of First FR	
Treatment	n	MDMA Mean (SEM)	Saline Mean (SEM)
(+)-Fenfluramine	11	53.1 (11.1)	11.8 (6.9)
Saline	10	89.9 (7.9)	23.5 (10.2)

TABLE 2

RESULTS OF SUBSTITUTION TESTS

Drug	Dose (mg/kg)	Drug-Appropriate Responding (%) Mean (SEM)				Response Rate (Responses per Second) Mean (SEM)	
		n/N*	Pretreatment	n/N*	Posttreatment	Pretreatment	Posttreatment
				Saline-Treated Animals			
MDMA	0.19	10/10	20.5 (13.3)	9/9	11.1 (11.1)	1.8 (0.2)	1.5 (0.1)
	0.38	10/10	20.3 (10.2)	9/9	0.3 (0.3)	1.5 (0.2)	1.5 (0.3)
	0.75	10/10	33.9 (13.3)	9/9	57.0 (16.2)	1.4 (0.2)	1.0 (0.3)
	1.50	10/10	100.0 (0.0)	8/8	100.0 (0.0)	1.4 (0.4)	0.6 (0.1)
(+)-Amphetamine	0.125	10/10	13.3 (9.4)	9/9	7.8 (3.8)	1.3 (0.2)	0.9 (0.2)
	0.25	10/10	1.4 (0.9)	9/9	15.1 (11.1)	0.8 (0.2)	1.1 (0.2)
	0.50	9/9	23.5 (14.5)	8/8	25.0 (15.2)	0.6 (0.2)	0.8 (0.3)
	1.0	9/10	37.0 (13.0)	7/8	47.8 (17.7)	0.2 (0.1)	0.1 (0.0)
Saline (1 ml/kg)	–	9/9	0.0 (0.0)	8/8	1.7 (1.2)	1.4 (0.2)	1.3 (0.3)
				(+)-Fenfluramine-Treated Animals			
MDMA	0.19	11/11	10.1 (8.2)	11/11	48.5 (14.9)	1.6 (0.3)	1.5 (0.2)
	0.38	11/11	27.7 (14.0)	10/10	29.3 (11.1)	1.5 (0.3)	1.4 (0.2)
	0.75	11/11	38.9 (14.8)	9/9	45.0 (17.4)	1.5 (0.3)	1.6 (0.3)
	1.50	11/11	99.6 (0.4)	8/8	100.0 (0.0)	1.0 (0.1)	0.8 (0.1)
(+)-Amphetamine	0.125	11/11	3.0 (1.4)	11/11	30.2 (13.4)	1.5 (0.2)	1.8 (0.2)
	0.25	10/10	8.6 (4.5)	9/9	52.2 (16.6)	1.1 (0.2)	1.5 (0.2)
	0.50	10/10	28.7 (12.8)	9/9	44.5 (15.4)	0.7 (0.2)	0.7 (0.3)
	1.0	10/11	38.8 (12.2)	5/8	96.2 (2.9)	0.5 (0.2)	0.1 (0.0)
Saline (1 ml/kg)	–	11/11	1.2 (1.2)	8/8	0.7 (0.7)	2.1 (0.4)	1.5 (0.4)

*Number of rats to complete FR/number of rats tested.

ment tests was unequal because five animals died prior to the completion of the study. The cause of death was believed to be unrelated to the (+)-fenfluramine treatment because two of the saline-treated and three of the (+)-fenfluramine-treated animals died.

A two-way repeated measures ANOVA on the MDMA dose–response test indicated a significant effect of dose on percent drug-appropriate responses in both fenfluramine-treated, $F(4, 49) = 25.87$, $p < 0.0001$, and saline-treated animals, $F(4, 45) = 39.34$, $p < 0.0001$. However, there was no significant effect of time and no significant dose × time interaction on percent drug-appropriate responding in either treatment group. A significant effect of MDMA dose on response rate was also noted in fenfluramine-treated, $F(4, 49) = 2.65$, $p < 0.05$, animals, although the time factor and dose × time interaction was not significant. The MDMA dose effect on response rate in saline-treated animals approached statistical significance, $F(4, 45) = 2.42$, $p = 0.06$, and there was a significant time effect on response rate in these animals, $F(1, 40) = 5.27$, $p < 0.05$, but no significant dose × time interaction.

As noted in Table 2, pretreatment substitution tests with (+)-amphetamine produced less than 40% MDMA-appropriate responding in both treatment groups. Although there was little change in the percent drug-appropriate responding elicited by (+)-amphetamine in the saline-treated animals during posttreatment substitution tests, (+)-fenfluramine–treated animals exhibited a greater amount of MDMA-appropriate responding with all doses of (+)-amphetamine. Moreover, the highest dose of (+)-amphetamine (1.0 mg/kg) substituted completely for MDMA subsequent to (+)-fenfluramine treatment and retraining on MDMA. A two-way repeated measures ANOVA on the (+)-amphetamine dose–response test revealed a significant dose effect, $F(4, 50) = 10.87$, $p < 0.0001$, and time effect, $F(1, 37) = 19.61$, $p < 0.0001$, on percent drug-appropriate responding in the fenfluramine-treated animals. The dose × time interaction approached statistical significance, $F(4, 37) = 2.44$, $p = 0.06$. In contrast, statistical analysis of percent drug-appropriate responding with (+)-amphetamine in saline-treated animals revealed a significant dose effect, $F(4, 44) = 4.95$, $p < 0.01$, but no significant time effect or dose × time interaction.

Higher doses of (+)-amphetamine also significantly lowered response rate in both fenfluramine-treated and saline-treated animals. The dose effect on response rate was statistically significant in fenfluramine-treated, $F(4, 50) = 14.44$, $p < 0.0001$, and saline-treated animals, $F(4, 44) = 10.06$, $p < 0.0001$, but there was no significant time effect or dose × time interaction on response rate in either group.

DISCUSSION

Several investigators have suggested that the discriminative stimulus properties of MDMA are complex, mediated by a combination of serotonergic and dopaminergic mechanisms (9,15,33). Because the substitution between MDMA and dopaminergic agents such as (+)-amphetamine and (−)-cathinone appears to be asymmetrical, it has been suggested that dopaminergic mechanisms comprise a weak component of MDMA's cue properties (32,33). In contrast, cross-substitution occurs between MDMA and 5-HT releasers (32,33). Moreover, the

5-HT_2 antagonists pirenpirone (33) and 5-HT_3 antagonists LY 27854 and zacopride (15) have been reported to significantly block MDMA discrimination, while the DA antagonist haloperidol appears to have relatively little effect on the MDMA cue (15,33). Other evidence in support of MDMA's compound stimulus effects are findings that MDMA substitutes for (±)-fenfluramine and (+)-amphetamine in pigeons trained to discriminate both these compounds in a three-choice procedure (9).

The present results from pretreatment substitution tests confirmed previous findings (33) that (+)-amphetamine does not substitute for MDMA (1.5 mg/kg). Oberlander and Nichols (30) have reported (+)-amphetamine (1.2 mg/kg) to substitute for a higher training dose of MDMA (1.75 mg/kg). However, that dose of (+)-amphetamine disrupted more than half of the animals tested, and higher doses did not substitute for MDMA. Additional methodological differences between the present study and that of Oberlander and Nichols (30) were the FR requirement (20 vs. 50, respectively) and the duration of test sessions (maximum 30 min vs. maximum 5 min, respectively). The extent to which these and other methodological differences contribute to conflicting results between laboratories should be examined systematically.

Glennon and Higgs (11) have argued that while there are some behavioral similarities between MDMA and (+)-amphetamine, the stimulus effects of MDMA are clearly distinct from those of (+)-amphetamine. For example, drugs that do not mimic (+)-amphetamine have been shown to substitute for MDMA [e.g., MDE, (12,14)] and, as mentioned above, the dopamine antagonist haloperidol blocks the (+)-amphetamine cue but does not completely antagonize the MDMA cue (15,33). If dopaminergic mechanisms are at least a partial component of the complex stimulus effects of MDMA, then serotonergic depletion might unmask or strengthen these mechanisms, making them more salient in mediating the interoceptive cue properties of MDMA.

Previous investigations have revealed that fenfluramine-induced neurotoxicity may have functional consequences that can be detected using drug discrimination procedures (34). Schechter (34) demonstrated that a presumably neurotoxic regimen of (±)-fenfluramine (26) enhanced the discrimination of lower doses of this compound and suggested that these effects were due to the development of postsynaptic supersensitivity. The present results suggest that a similar regimen of (+)-fenfluramine treatment may also increase sensitivity to low doses of MDMA. In posttreatment tests of (+)-fenfluramine-treated animals, 0.19 mg/kg of MDMA elicited greater drug-appropriate responding (48%) than in pretreatment tests with this dose (10%); however, the factor of time (pretreatment vs. posttreatment) was not statistically significant.

While the present study was in progress, Schechter (35) demonstrated that a neurotoxic regimen of MDMA (20 mg/kg SC twice a day for 4 days) actually diminished the interoceptive cue properties of low doses (0.5 to 1.5 mg/kg) of MDMA. Those investigations employed a unique testing procedure that did not allow for retraining after the neurotoxic MDMA regimen. Although, in the present study, discrimination training was resumed prior to administering posttreatment substitution tests, a review of the data from the first few training sessions revealed some interesting results that are not inconsistent with Schechter's (35) findings. The data illustrated in Table 1 suggest that the interoceptive cue associated with MDMA was diminished by (+)-fenfluramine treatment, at least at the onset of resuming discrimination training. Although many of the (+)-fenfluramine-treated animals initially made less than 80% of their responses on the drug-appropriate lever upon resuming MDMA discrimination training, with subsequent training, discrimination performance improved and testing criteria were met within 4 to 11 training sessions. The present findings, therefore, suggest that although the (+)-fenfluramine regimen may have diminished serotonergic mechanisms mediating the stimulus effects of MDMA, animals were still capable of discriminating MDMA from saline. Thus, it is possible that dopaminergic mechanisms played a significant role in reestablishing the discrimination of MDMA in (+)-fenfluramine-treated animals.

Because (+)-amphetamine substituted completely for MDMA following (+)-fenfluramine treatment, but not following saline treatment, perhaps serotonergic changes induced by (+)-fenfluramine enhanced the dopaminergic mediation of MDMA's stimulus effects. This interpretation is consistent with previous findings that serotonin depletion induced by neurotoxic doses of MDMA enhanced the psychomotor stimulant effects of acute MDMA administration on DRL schedule-controlled responding (27).

Schechter has demonstrated in several experiments (34–36) that exposure to drug regimens known to produce serotonin depletion cause functional changes that are easily detected using drug discrimination procedures. Moreover, these procedures have been useful in determining the importance of 5-HT mechanisms in mediating the stimulus properties of MDMA. Although the reversible 5-HT synthesis inhibitor, *p*-CPA was shown to only temporarily attenuate MDMA discrimination (36), the presumably neurotoxic compounds, fenfluramine and MDMA, appear to have longer lasting effects (34,35). Schechter examined the effects of serotonin depletion on the discrimination of (±)-fenfluramine (34) and MDMA (35) discrimination 2 weeks after the initiation of the neurotoxic dose regimens. The extent to which these effects are permanent must be examined using a longer time course, although maintaining an accurate discrimination may be difficult with a longer period of extinction from training.

In the present study, several doses of MDMA and (+)-amphetamine were assessed over a 10- to 14-week period following (+)-fenfluramine or saline administration. Previous examination of the neurotoxic effects of (±)-fenfluramine have indicated that administration of 12 mg/kg/day for 4 days depletes 5-HT and 5-HIAA for up to 6 months after cessation of drug treatment (20,26). Because serotonin neurotoxicity is attributed to (+)-fenfluramine and not to (−)-fenfluramine (24), the dose of (+)-fenfluramine used in the present study (8 mg/kg/day for 4 days) was slightly lower than the dose of (±)-fenfluramine reported to produce prolonged serotonergic deficits. However, because neurochemical analyses were not conducted at the conclusion of the present study, an interpretation that the observed changes in the discriminative stimulus properties of MDMA were related to serotonin depletion is merely speculative at this point. Additional neuropharmacological investigations on the long-term consequences of high-dose (+)-fenfluramine administration in animals maintained on chronic low dose MDMA administration are required to confirm such speculations.

Given the dynamic, mutable nature of the central nervous system, a complete understanding of the neuronal mechanisms of drug action requires the employment of sensitive behavioral measures. Although the drug discrimination paradigm has proven a reliable and sensitive tool to investigate the underly-

ing mechanisms of psychoactive drug effects, most psychoactive drugs produce multiple actions in the CNS and may, therefore, have complex stimulus effects. Although the importance of serotonergic mechanisms underlying the discriminative stimulus effects of MDMA is well documented (1,15,33), the role of dopaminergic mechanisms is still under investigation. Methods that employ pharmacological manipulations, such as 5-HT depletion in this and previous studies (35,36), have revealed that the stimulus effects of MDMA are not immutable. Because MDMA is potentially neurotoxic (37) and DA release plays a major role in its neurotoxicity (39), continued investigations on dopaminergically mediated behavioral effects of MDMA are imperative.

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