

Serotonin Release Contributes to the Locomotor Stimulant Effects of 3,4-Methylenedioxymethamphetamine in Rats¹

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ABSTRACT

Methylenedioxymethamphetamine (MDMA) is a phenylethylamine with novel mood-altering properties in humans. MDMA shares the dopamine-releasing properties of amphetamine but has been found to be a more potent releaser of serotonin (5-HT). The present study undertook to determine the relative roles of dopamine and 5-HT release in MDMA-induced locomotor hyperactivity. S-(+)-MDMA produced dose-dependent increases of rat locomotion. Investigatory behaviors such as holepokes and rearings were suppressed by (+)MDMA. Pretreatment with the selective 5-HT uptake inhibitors fluoxetine, sertraline and zimelidine inhibited (+)MDMA-induced locomotor hyperactivity but failed to antagonize the reduction of holepokes and rearings. Because 5-HT uptake inhibitors have been found previously to

block the MDMA-induced release of 5-HT *in vitro*, and because fluoxetine was found to have no effect on (+)amphetamine-induced hyperactivity, the present results suggest that (+)MDMA-induced locomotor hyperactivity is dependent on release of endogenous 5-HT. Additionally, prior depletion of central 5-HT with *p*-chlorophenylalanine partially antagonized the (+)MDMA-induced hyperactivity, although catecholamine synthesis inhibition with *alpha*-methyl-*p*-tyrosine did not block the effects of (+)MDMA. Taken together, these studies suggest that (+)MDMA increases locomotor activity via mechanisms that are dependent on the release of central 5-HT and that are qualitatively different from the mechanism of action of (+)amphetamine.

MDMA is structurally related to amphetamine but is distinguished by novel psychological effects in humans (Anderson *et al.*, 1978). With a behavioral profile resembling both psychostimulants and hallucinogens in a variety of animal paradigms (Gold *et al.*, 1988; Glennon and Young, 1984), MDMA has been proposed to be a member of a novel drug class (Nichols *et al.*, 1986). Biochemical evidence indicates that MDMA shares the DA-releasing properties of amphetamine both *in vitro* (Johnson *et al.*, 1986; Schmidt *et al.*, 1987) and *in vivo* (Yamamoto and Spanos, 1988). However, MDMA is much more potent than amphetamine at releasing 5-HT, prompting the suggestion that the unique behavioral effects of MDMA result from 5-HT release (Steele *et al.*, 1987). The long-lasting 5-HT depletion induced by MDMA (Schmidt, 1987) as well as the effects of MDMA on single cell firing (Sprouse and Aghajanian, 1988) and neuroendocrine function (Nash *et al.*, 1988) have been linked to 5-HT release. However, the contribution of serotonergic mechanisms to the stimulant-like effects of MDMA have not been studied adequately.

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In rats, MDMA induces hyperactivity consisting primarily of increased forward locomotion (Gold *et al.*, 1988; Spanos and Yamamoto, 1989). Because of extensive evidence that the locomotor-activating properties of amphetamine result from central DA release (Kelly *et al.*, 1975), it has been suggested that the stimulant properties of MDMA also may be mediated by DA release (Gold *et al.*, 1988; Gold and Koob, 1988). Indeed, lesions of the mesolimbic DA system with 6-hydroxydopamine have been shown to interfere with MDMA-induced locomotor activity in rats (Gold *et al.*, 1989). However, the pattern of locomotor activity induced by MDMA is qualitatively different from the pattern of activity induced by doses of amphetamine that produce similar increases of locomotion (Gold *et al.*, 1988). Furthermore, MDMA decreases investigatory behaviors that are increased by amphetamine and other DA-releasing agents. Hence, behavioral stimulation after MDMA and amphetamine may represent qualitatively different syndromes. Consequently, even though both MDMA and amphetamine increase the quantity of activity in rats, it is premature to conclude that these increases are mediated by the same neurochemical mechanism.

The present study addresses the possibility that 5-HT release is important for (+)MDMA-induced locomotor hyperactivity in rats. Because monoamine release by amphetamine-like drugs

ABBREVIATIONS: MDMA, 3,4-methylenedioxymethamphetamine; 5-HT, 5-hydroxytryptamine (serotonin); DA, dopamine; PCA, *p*-chloro-amphetamine; PCPA, *p*-chloro-phenylalanine; AMPT, *alpha*-methyl-*p*-tyrosine; BPM, behavioral pattern monitor; CV, coefficient of variation; i.p., intraperitoneal.

requires functional monoamine uptake systems (Fischer and Cho, 1979; Raiteri *et al.*, 1979) and fluoxetine selectively inhibits 5-HT uptake (Wong *et al.*, 1975), fluoxetine pretreatment similar to that previously reported to antagonize other effects of MDMA (Nash *et al.*, 1988; Schmidt, 1987) was employed to block MDMA-induced 5-HT release without interfering with DA release. Fluoxetine was found to attenuate MDMA-induced locomotor hyperactivity. Similar reductions were observed after 5-HT synthesis inhibition but not catecholamine synthesis inhibition. These data indicate that the locomotor hyperactivity induced by (+)MDMA is dependent on the 5-HT-releasing properties of this drug.

Methods

Animals. Male Sprague-Dawley rats (Harlan, CA) weighing 300 to 400 g were used in all experiments. Animals were housed two per cage with food and water available *ad libitum*. Lighting was on a reverse light/dark cycle (12 h/12 h). All rats were allowed at least 1 week of acclimation after arrival before experiments and were tested during the dark phase of the light/dark cycle.

Behavioral measures. Behavior was measured in a BPM system designed to monitor behavioral patterns as described previously (Flicker and Geyer, 1982; Geyer *et al.*, 1986). Briefly, the BPM consists of a 30.5 × 61.0 cm chamber with smooth metal floor and opaque Plexiglas walls. Each long wall has three 2.5-cm diameter holes placed at equal intervals 2.5 cm above the floor. Three additional holes are placed in the long axis of the floor, and one hole is situated in the center of one short wall. A 4 × 8 grid of infrared photobeams is projected through the BPM allowing the X-Y position of the animal to be localized with a resolution of 3.8 cm. Photocells located in each of the wall and floor holes allow detection of investigatory nosepokes (holepokes). Finally, a metal touchplate located 15.2 cm above the floor allows detection of animal rearings against the wall. A computer continuously monitors the status of all photobeams and records the duration and nature of the changes.

Analysis. The number of crossings between any of eight square regions was calculated from the raw data as an index of the quantity of locomotor activity. Numbers and durations of holepokes and rearings were calculated directly from the raw data. These analyses were similar to those described previously (Geyer *et al.*, 1986; Gold *et al.*, 1988) but were based on new computer programs written in C and run on an IBM-PC compatible computer. The "crossings" measure in this study is based on more stringent requirements for the definition of movement between squares than the previously used measure of "crossovers" (Geyer *et al.*, 1986). Although the two measures covary consistently, fewer crossings than crossovers are scored. The raw data from a test session could also be plotted to reconstruct the path of the animal within the BPM. These plots allowed recognition of the characteristic patterns of locomotor activity elicited by the different drugs. Furthermore, a descriptive statistic, the CV9, was calculated from the number of particular region transitions made by the animal (Geyer *et al.*, 1986; Gold *et al.*, 1988). The CV9 provides an index of the diversity of the path followed by the animal within the BPM, with increasing values of the CV9 indicating that some paths or region transitions were repeated preferentially.

Crossings, holepokes and rearings were initially examined in 10-min intervals. Statistical comparisons were performed using repeated measures analysis of variance (Dixon, 1988). Posthoc comparisons were made using Tukey's Studentized Range Method on 30- or 60-min samples of data.

Drugs. Drugs used were (+)MDMA HCl (NIDA, Rockville, MD) (0.3, 1.0, 3.0, 10.0 mg/kg), (+)amphetamine SO₄ (Sigma, St. Louis, MO) (2.0 mg/kg), fluoxetine (Lilly, Indianapolis, IN) (10.0 mg/kg), PCPA (Sigma) (200 mg/kg), AMPT (Lilly) (125 mg/kg), chlorimipramine HCl (CIBA-Geigy, Basel, Switzerland) (10.0 mg/kg), sertraline (Pfizer, Groton, CN) (10.0 mg/kg) and zimelidine (Astra, Sodertälje,

Sweden) (10.0 mg/kg). Doses are based on salt weight of the drug except for (+)amphetamine and PCPA for which free-base doses are given. (+)MDMA, (+)amphetamine, chlorimipramine, sertraline and zimelidine were given by injection in a volume of 1.0 ml/kg. Fluoxetine, PCPA and AMPT were given by injection in a volume of 2.0 ml/kg. The vehicle for all drugs except sertraline was 0.9% saline. Sertraline was dissolved in a solution of propylene glycol, ethanol and acetic acid (10:40:50).

Experimental procedure. Animals were brought from the animal colony in individual cages at least 1 h before testing. (+)MDMA and (+)amphetamine were administered s.c. 10 min before placing the animal in the BPM. Fluoxetine, sertraline, zimelidine and chlorimipramine were administered i.p. 60 min before testing, and AMPT was administered i.p. 120 min before testing. PCPA was administered i.p. while the animal was in its home cage 72 h before test sessions. Control subjects for each drug treatment received equivalent volumes of 0.9% saline at the same time *via* the same routes. Control groups were shared for the PCPA and AMPT studies, which were run concurrently. After the animal was placed in the BPM, data were collected for 1 h.

Results

Dose-response for (+)MDMA. A significant effect of (+)MDMA dose on crossings was found ($F = 32.64$; $df = 4,50$; $P < .0001$). The greatest levels of locomotor hyperactivity occurred after 3.0 and 10.0 mg/kg of (+)MDMA. As displayed in figure 1, there was a significant effect of time on crossings ($F = 62.14$; $df = 5,250$; $P < .0001$) and a significant interaction between drug and time ($F = 14.01$; $df = 20,250$; $P < .0001$). Levels of activity in control animals decreased during the session as the animal adapted to the novel testing environment. In contrast, (+)MDMA produced a sustained increase in crossings. Holepokes were examined initially as repeated holepokes (pokes occurring at the same hole during a single bout of investigation) and as varied holepokes (the initial poke in a bout of investigation). Because no differences were observed in the drug effects on these two measures, only total holepokes are presented in these and subsequent analyses. As detailed in table 1, (+)MDMA decreased the number of holepokes made by the rats after 1.0 mg/kg and greater doses ($F = 11.10$; $df = 4,50$; $P < .0001$). Rearings were depressed at the same doses ($F = 43.97$; $df = 4,50$; $P < .0001$; table 1). Animals receiving 3.0 and 10.0 mg/kg (+)MDMA were observed to have polyuria and occasionally left ejaculatory plugs in the BPM.

(+)MDMA induced a characteristic pattern of locomotion as illustrated in figure 2. Animals typically moved along the walls of the BPM and avoided the center of the chamber, creating a pattern of locomotion superficially similar to the rotational behavior produced by the direct DA agonist apomorphine (Geyer *et al.*, 1986). Unlike apomorphine-induced rotation, however, an individual animal treated with (+)MDMA did not exhibit a preferential direction of rotation about the chamber but often alternated directions. The effect of (+)MDMA on spatial patterns of locomotion is also reflected by a dose-dependent increase of the CV9 calculated over the entire 60-min session ($F = 3.95$; $df = 4,50$; $P < .05$). The mean $\pm$ S.E.M. CV9 after saline, 0.3, 1.0, 3.0 or 10.0 mg/kg, (+)MDMA were 1.033 ± 0.023 , 1.026 ± 0.051 , 0.995 ± 0.025 , 1.018 ± 0.041 and 1.202 ± 0.057 , respectively. Posthoc comparisons of these values revealed that only after the 10.0-mg/kg dose of (+)MDMA was the CV9 significantly different from control values ($P < .05$).

The 3.0-mg/kg dose of (+)MDMA produced robust stimulation of locomotor activity as well as significant reductions of rearings and holepokes. These behavioral effects were sustained

(+) MDMA Dose-Response

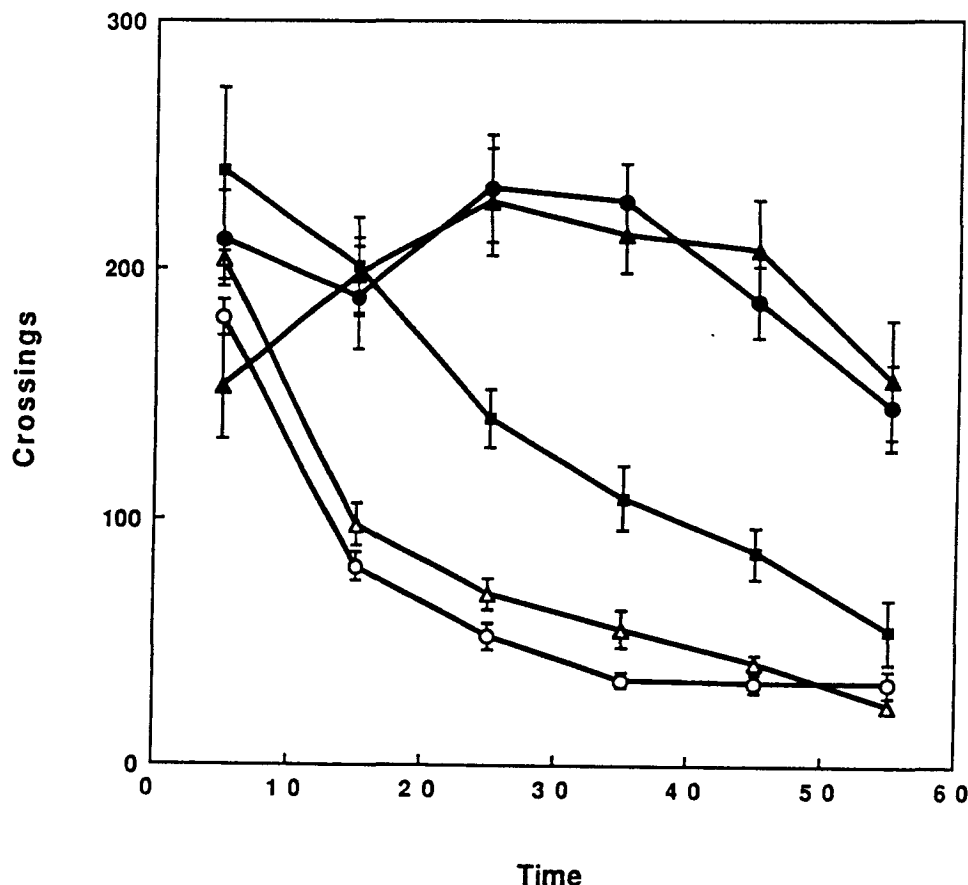


Fig. 1. Dose-dependent effect of (+)MDMA on crossings. Each point represents the mean $\pm$ S.E.M. number of crossings per 10-min interval. Saline ($n = 12$) (○) or (+)MDMA was administered s.c. 10 min before testing. Doses (mg/kg) were 0.3 ($n = 11$) (△), 1.0 ($n = 9$) (■), 3.0 ($n = 11$) (●) and 10.0 ($n = 12$) (▲).

TABLE 1

Dose-dependent effect of (+)MDMA on behavioral measures

(+)MDMA was administered s.c. 10 min before testing. Values are mean $\pm$ S.E.M. per 30-min interval.

Dose (n) mg/kg	0.0 (12)	0.3 (11)	1.0 (9)	3.0 (11)	10.0 (12)
Crossings					
0-30 min	310.7 $\pm$ 14.6	367.7 $\pm$ 18.0	566.1 $\pm$ 52.7**	632.6 $\pm$ 50.3**	575.6 $\pm$ 52.3**
30-60 min	98.9 $\pm$ 7.1	118.8 $\pm$ 10.0	246.6 $\pm$ 28.8*	556.8 $\pm$ 36.9**	575.6 $\pm$ 52.6**
Holepokes					
0-30 min	108.6 $\pm$ 10.1	95.1 $\pm$ 11.2	39.8 $\pm$ 5.4**	32.6 $\pm$ 3.3**	36.7 $\pm$ 3.2**
30-60 min	72.7 $\pm$ 14.9	78.7 $\pm$ 13.0	39.4 $\pm$ 6.9	36.5 $\pm$ 5.6	34.7 $\pm$ 14.3
Rearings					
0-30 min	102.1 $\pm$ 11.9	89.5 $\pm$ 7.5*	31.3 $\pm$ 6.5**	0.8 $\pm$ 0.5**	0.3 $\pm$ 0.2**
30-60 min	31.3 $\pm$ 4.9	34.7 $\pm$ 4.4	39.8 $\pm$ 7.4	11.7 $\pm$ 2.7*	0.4 $\pm$ 0.3**

* $P < .05$; ** $P < .01$ vs. saline alone.

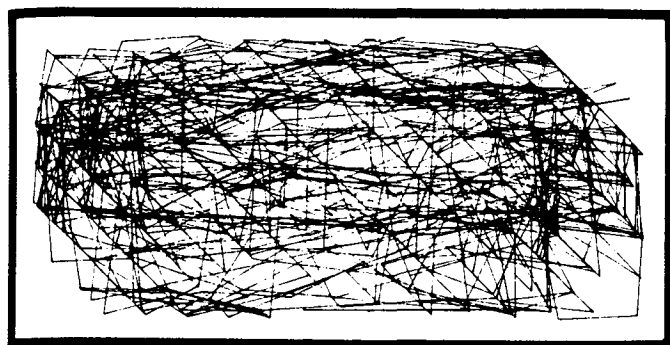
throughout the 60-min test session. Consequently, 3.0 mg/kg of (+)MDMA were used for subsequent interaction studies. The 3.0-mg/kg dose of (+)MDMA was unsuitable, however, for making meaningful comparisons of the spatial pattern of locomotor activity using the CV9 statistic. Therefore, the CV9 is not considered in subsequent experiments.

Fluoxetine pretreatment. Fluoxetine has been found previously not to affect the locomotor activity of rats at the dose used in this study (Geyer, unpublished observations). The present data confirm that fluoxetine had no significant effect by itself on crossings. However, there was a significant interaction between fluoxetine pretreatment and (+)MDMA ($F = 15.42$; $df = 1,36$; $P < .001$), reflecting the fact that the number of crossings induced by (+)MDMA was reduced significantly in animals pretreated with fluoxetine (fig. 3A). Fluoxetine alone

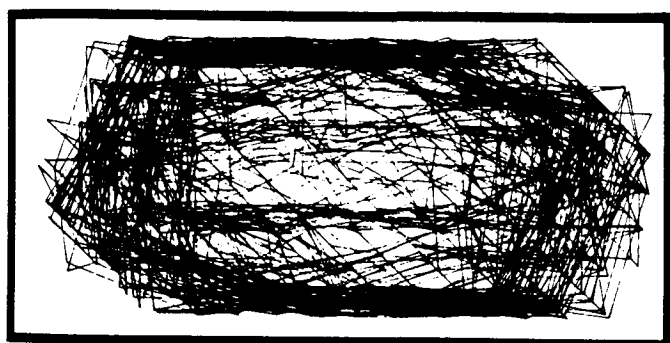
produced a nonsignificant reduction of holepokes and a significant reduction of rearings ($P < .01$). Holepokes were further suppressed by (+)MDMA in fluoxetine-pretreated animals (fig. 3B). A significant interaction between pretreatment and drug for rearings ($F = 7.73$; $df = 1,36$; $P < .01$) reflects the fact that (+)MDMA reduced rearings to a lesser extent in fluoxetine-pretreated animals than in saline-pretreated animals (fig. 3C). Inspection of the plots of locomotor paths revealed that fluoxetine pretreatment increased the proportion of time spent in the center of the BPM by (+)MDMA-treated rats (fig. 2C).

In order to test for possible nonspecific effects of fluoxetine pretreatment on locomotor activity as well as interactions between fluoxetine and DA systems, the effect of fluoxetine on (+)amphetamine-induced locomotor hyperactivity was examined. As shown in table 2, 2.0 mg/kg of (+)amphetamine

A. Saline



B. (+)MDMA



C. Fluoxetine and (+)MDMA

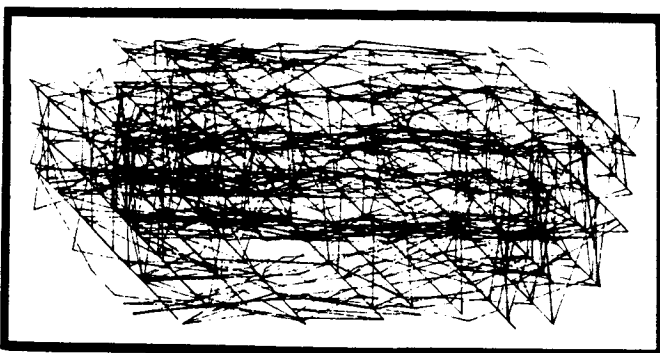


Fig. 2. Computer reconstruction of the locomotor path followed during 60-min test session by individual rats receiving (A) saline alone, (B) 3.0 mg/kg of (+)MDMA alone or (C) 10.0 mg/kg of fluoxetine and 3.0 mg/kg of (+)MDMA before being placed in the BPM. (+)MDMA increases the quantity of activity and decreases entries into the center of the chamber.

significantly increased crossings ($F = 15.92$; $df = 1,28$; $P < .001$). This effect was not altered by fluoxetine as indicated by the lack of a significant interaction between amphetamine and fluoxetine. (+)Amphetamine produced nonsignificant increases in holepokes and rearings at this dose. A significant interaction

Fluoxetine Pretreatment

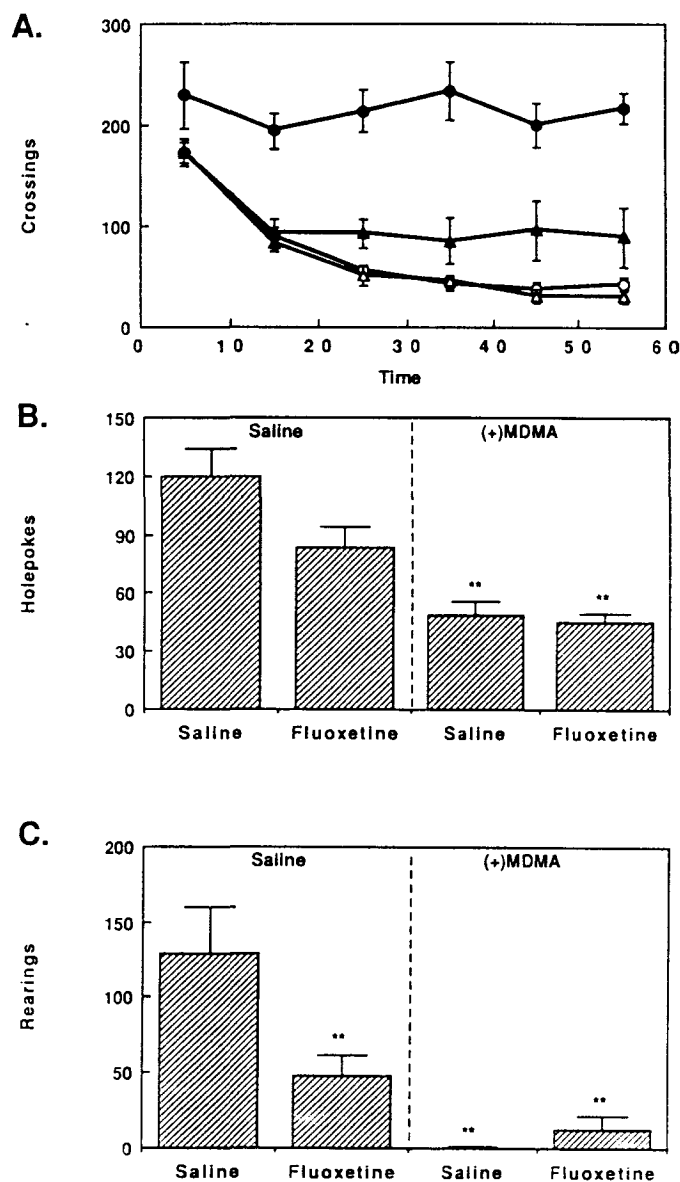


Fig. 3. Interaction between fluoxetine (i.p. 60 min before testing) and (+)MDMA (s.c. 10 min before testing). (A) Crossings are presented as in figure 1. Pretreatment/treatment groups are saline/saline ($n = 10$) (○), fluoxetine/saline ($n = 10$) (△), saline/(+)MDMA ($n = 10$) (●) and fluoxetine/(+)MDMA ($n = 10$) (▲). (B) Effect of fluoxetine and (+)MDMA on holepokes, presented as mean $\pm$ S.E.M. per 60-min test session. (C) Effect of fluoxetine and (+)MDMA on rearings, presented as mean $\pm$ S.E.M. per 60-min test session. ** Significantly different from saline/saline control group, $P < .01$.

between fluoxetine, amphetamine and time for rearings ($F = 4.18$; $df = 5,140$; $P < .01$) indicated that fluoxetine partially antagonized the increase in rearings induced by amphetamine during the second half of the session (table 2). No significant interactions between fluoxetine and (+)amphetamine were observed for holepokes.

Other 5-HT uptake inhibitors shared the ability of fluoxetine to antagonize (+)MDMA-induced hyperactivity, as summarized in figure 4. Sertraline had a significant interaction with MDMA on crossings ($F = 8.56$; $df = 1,25$; $P < .01$) because of its ability to reduce (+)MDMA-induced increases in this measure. Although zimelidine did not have a significant simple interaction with (+)MDMA, there was a significant interaction

TABLE 2

Interaction between fluoxetine and (+)amphetamine

Fluoxetine (10.0 mg/kg) was administered i.p. 60 min before testing; (+)amphetamine (2.0 mg/kg) was administered s.c. 10 min before testing. Values are mean $\pm$ S.E.M. per 30-min interval.

Pretreatment/ Treatment (n)	Saline/Saline (8)	Fluoxetine/Saline (8)	Saline/ Amphetamine (8)	Fluoxetine/ Amphetamine (8)
Crossings				
0-30 min	346.9 $\pm$ 26.6	311.9 $\pm$ 29.6	583.6 $\pm$ 80.8*	495.4 $\pm$ 67.4
30-60 min	134.6 $\pm$ 23.4	126.5 $\pm$ 12.8	465.9 $\pm$ 102.0**	312.0 $\pm$ 87.0
Holepokes				
0-30 min	104.1 $\pm$ 7.0	53.4 $\pm$ 10.2	102.4 $\pm$ 60.3	44.0 $\pm$ 12.5
30-60 min	71.4 $\pm$ 13.4	19.8 $\pm$ 4.5	155.1 $\pm$ 94.5	28.3 $\pm$ 17.0
Rearings				
0-30 min	88.0 $\pm$ 10.3	50.6 $\pm$ 9.9	65.4 $\pm$ 19.6	55.4 $\pm$ 42.6
30-60 min	29.9 $\pm$ 7.7	23.3 $\pm$ 3.9	145.8 $\pm$ 48.9*	42.8 $\pm$ 26.5

* $P < .05$; ** $P < .01$ vs. saline/saline group.

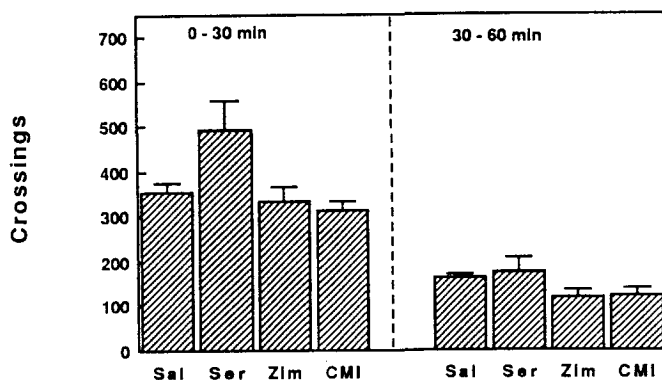
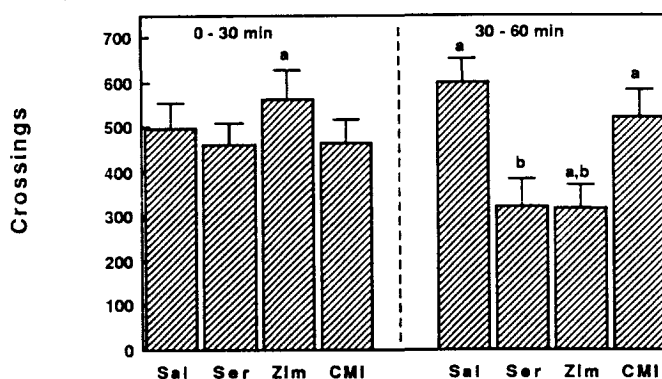
5HT-Uptake Inhibitor Pretreatment**A. Saline****B. (+)MDMA**

Fig. 4. Interaction of 5-HT uptake inhibitors with (+)MDMA. Saline (Sal), sertraline (Ser), zimelidine (Zim) or chlorimipramine (CMI) was administered i.p. 60 min before testing. Animals were placed in the BPM 10 min after s.c. injections of (A) saline or (B) (+)MDMA. Mean $\pm$ S.E.M. numbers of crossings per 30-min interval are presented. All groups contained six to seven animals. (a, pretreatment/treatment group is significantly different from saline/saline control group, $P < .05$; b, pretreatment/treatment group is significantly different from corresponding saline/treatment group, $p < .05$).

between zimelidine, (+)MDMA and time ($F = 11.02$; $df = 5,140$; $P < .0001$). Inspection of the data in 30-min intervals (fig. 4) revealed that zimelidine did not antagonize the effect of (+)MDMA during the 0- to 30-min interval, but it did reduce (+)MDMA-induced hyperactivity between 30 and 60 min ($F = 9.84$; $df = 1,28$; $P < .01$). Chlorimipramine did not affect MDMA-induced increases in activity.

Synthesis inhibitors. PCPA pretreatment decreased crossings ($F = 9.90$; $df = 1,31$; $P < .01$) in both saline- and (+)MDMA-treated animals (fig. 5), but the two-way interaction between PCPA and (+)MDMA was not significant. A significant interaction between PCPA, (+)MDMA and time ($F = 8.20$; $df = 5,155$; $P < .0001$), however, prompted separate analyses of crossings from 0 to 30 min and from 30 to 60 min. Although PCPA did not significantly reduce crossings from 0 to 30 min in either group, the (+)MDMA-induced increase in crossings between 30 and 60 min was significantly attenuated in PCPA-pretreated animals ($F = 7.93$; $df = 1,31$; $P < .01$). A significant interaction between PCPA and (+)MDMA for rearings ($F = 16.08$; $df = 1,31$; $P < .001$) indicated that PCPA also antagonized the reduction of rearings induced by (+)MDMA (data not shown). However, PCPA did not affect investigatory holepokes or the suppression of holepokes by (+)MDMA (data not shown).

In contrast, AMPT pretreatment reduced crossings ($F = 4.67$; $df = 1,30$; $P < .05$), investigatory holepokes ($F = 8.72$; $df = 1,30$; $P < .01$) and rearings ($F = 5.22$; $df = 1,30$; $P < .05$). No significant interactions between AMPT and (+)MDMA were observed for crossings or holepokes, as shown in table 3. Furthermore, no significant interaction between AMPT, (+)MDMA and time was found. A significant interaction between AMPT and (+)MDMA for rearings ($F = 4.71$; $df = 1,30$; $P < .05$) reflects the fact that AMPT alone greatly suppressed rearings, and a further reduction of rearings by (+)MDMA was not possible.

Discussion

The present study raises the possibility that the central release of presynaptic 5-HT contributes to the stimulant-like effects of MDMA. (+)MDMA produces behavioral stimulation at comparable or lower doses than racemic MDMA. For example, the pattern of behavior after the 3.0-mg/kg dose of (+)MDMA closely resembles the pattern previously reported after 5.0 mg/kg of racemic MDMA (Gold *et al.*, 1988). Furthermore, (+)MDMA may be the psychologically more potent isomer in humans (Anderson *et al.*, 1978). We have also found that much higher doses of (-)MDMA are required for behavioral stimulation in rats (Geyer and Wing, unpublished data), suggesting that the stimulant properties of racemic MDMA reside primarily in (+)MDMA. (+)MDMA is more potent than (-)MDMA at 5-HT release (Nichols *et al.*, 1982), supporting the hypothesis that 5-HT release is important for behavioral effects of MDMA. However, (+)MDMA is also a more potent

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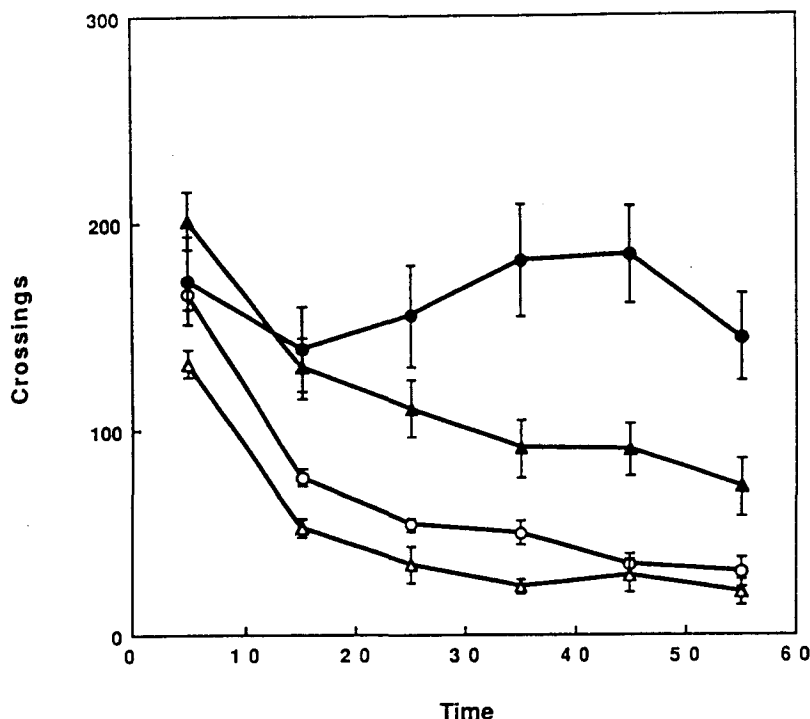


Fig. 5. Interaction between PCPA (i.p. 72 hours before testing) and (+)MDMA (s.c. 10 min before testing). (A) Crossings are presented as in figure 1. Pretreatment/treatment groups are saline/saline ($n = 8$) (○), PCPA/saline ($n = 9$) (△), saline/(+)MDMA ($n = 8$) (●) and PCPA/(+)MDMA ($n = 10$) (▲).

TABLE 3

Interaction between AMPT and (+)MDMA

AMPT (125 mg/kg) was administered i.p. 120 min before testing; (+)MDMA (3.0 mg/kg) was administered s.c. 10 min before testing. Values are mean $\pm$ S.E.M. per 30-min interval.

Pretreatment/ Treatment (n)	Saline/Saline (8)	AMPT/ Saline (8)	Saline/ (+)MDMA (8)	AMPT/ (+)MDMA (10)
Crossings				
0-30 min	294.3 $\pm$ 10.2	235.6 $\pm$ 21.3	465.3 $\pm$ 60.6*	437.0 $\pm$ 46.7
30-60 min	112.6 $\pm$ 13.1	72.0 $\pm$ 15.5	508.0 $\pm$ 68.6**	365.3 $\pm$ 21.1***
Holepokes				
0-30 min	73.6 $\pm$ 10.5	48.3 $\pm$ 5.2*	26.6 $\pm$ 4.6**	15.8 $\pm$ 7.6**
30-60 min	47.5 $\pm$ 10.6	20.5 $\pm$ 5.5	33.5 $\pm$ 5.6	24.6 $\pm$ 4.9
Rearings				
0-30 min	82.5 $\pm$ 17.1	44.4 $\pm$ 12.3	0.6 $\pm$ 0.7**	2.4 $\pm$ 1.1**
30-60 min	31.3 $\pm$ 8.4	7.6 $\pm$ 2.8*	14.0 $\pm$ 6.3	10.9 $\pm$ 4.1

* $P < .05$; ** $P < .01$ vs. saline/saline group; *** $P < .05$ vs. saline/(+)MDMA group.

DA-releasing agent than (-)MDMA (Johnson *et al.*, 1986). Consequently, the activity (+)MDMA does not rule out the possibility that DA release is responsible for the behavioral hyperactivity induced by MDMA.

The importance of 5-HT release for the behavioral effects of MDMA is supported primarily by the observation that fluoxetine reduces (+)MDMA-induced locomotor hyperactivity. The failure of fluoxetine pretreatment to affect (+)MDMA-induced reductions in holepokes provides evidence that fluoxetine did not interfere with the ability of (+)MDMA to reach the brain. Fluoxetine failed to affect the activity of control animals or to attenuate the response to (+)amphetamine, indicating that (+)MDMA-induced hyperactivity was not reduced by a non-specific effect of fluoxetine on motor performance. These data also confirm that fluoxetine pretreatment does not interfere with the drug-induced release of DA that putatively mediates the behavioral effect of amphetamine (Kelly *et al.*, 1975). Sertraline and zimelidine partially antagonized (+)MDMA-induced hyperactivity, confirming that fluoxetine shared the ability to antagonize (+)MDMA-induced hyperactivity with other

5-HT uptake inhibitors (Koe *et al.*, 1983; Ogren *et al.*, 1981). The tricyclic compound chlorimipramine did not reduce (+)MDMA-induced locomotor hyperactivity. Although chlorimipramine is an effective 5-HT uptake inhibitor, relative to the other 5-HT uptake inhibitors examined, chlorimipramine and its demethylated metabolite are less selective for 5-HT vs. catecholamine uptake blockade (Fuller *et al.*, 1974). Additionally, chlorimipramine has been shown to be less potent and to have a shorter duration of action than fluoxetine in the antagonism of 5-HT depletion by PCA (Fuller *et al.*, 1978). The dose of chlorimipramine used in the present study thus may have been too low for any antagonism of (+)MDMA-induced hyperactivity to be observed.

The data thus indicate that inhibition of 5-HT uptake antagonizes the locomotor hyperactivity induced by (+)MDMA. Release of monoamines from nerve terminals by amphetamine-like drugs is dependent on the functional integrity of the uptake carrier (Fischer and Cho, 1979; Raiteri *et al.*, 1979), and specific binding of (+)MDMA in the brain may be at the 5-HT uptake site (Gehlert *et al.*, 1985). Another 5-HT uptake inhibitor,

citalopram, has been shown to inhibit (+)MDMA-induced release of 5-HT *in vitro* (Schmidt *et al.*, 1987). Furthermore, pretreatment with doses of 5-HT uptake inhibitors similar to those used in the present study was found previously to block PCA-induced behavioral effects that are believed to be mediated by 5-HT release (Ogren and Johansson, 1985). Together, these data suggest that (+)MDMA-induced hyperactivity is dependent on the drug-induced release of 5-HT.

The shortened response to (+)MDMA in PCPA-pretreated animals provides further support for the hypothesis that (+)MDMA-induced hyperactivity is dependent on drug-induced 5-HT release, contrasting with the potentiation of amphetamine-induced locomotor hyperactivity reported to occur after PCPA pretreatment (Breese *et al.*, 1974; Hollister *et al.*, 1976; Mabry and Campbell, 1973; Segal, 1976). Acute administration of (+)MDMA has been shown previously to deplete 5-HT stores *in vitro* (Stone *et al.*, 1986; Schmidt, 1987), and blockade of new 5-HT synthesis by PCPA may have accelerated this depletion of releasable 5-HT. At the dose and pretreatment time used in the present study, PCPA is reported to produce a selective depletion of brain 5-HT without significantly reducing catecholamine levels (Koe and Weissman, 1966). The failure of AMPT pretreatment to attenuate the hyperactivity induced by (+)MDMA confirms that any catecholamine depletion that might have been produced by PCPA did not contribute to the abbreviated response to (+)MDMA. Similar AMPT pretreatment does antagonize (+)amphetamine-induced locomotor hyperactivity (Kuczenski, 1983; Scheel-Kruger, 1971). Thus, the present findings are also inconsistent with the hypothesis that MDMA produces stimulant-like effects *via* direct catecholamine release (Gold and Koob, 1988; Gold *et al.*, 1989), further supporting the conclusion that amphetamine and MDMA increase locomotion *via* different mechanisms. Alternatively, figure 3 suggests that (+)MDMA produces a small increase in locomotor activity in animals pretreated with fluoxetine, although this increase was not significant with the present sample. Conversely, 6-hydroxydopamine lesions in the mesolimbic DA system only partially attenuate the locomotor activity induced by MDMA (Gold *et al.*, 1989). It remains possible, therefore, that DA release and 5-HT release make additive contributions to the locomotor activating effects of (+)MDMA. Further studies with drugs having more selectivity for 5-HT *vs.* DA release should clarify the relative contributions of the two monoamines to the locomotor effects of the substituted amphetamines.

In contrast to its effects on locomotion, the effects of (+)MDMA on holepokes and rearings did not appear to depend on 5-HT release. In particular, fluoxetine and PCPA failed to reduce the effect of (+)MDMA on holepokes. Fluoxetine and PCPA did partially antagonize the suppressant effect of (+)MDMA on rearings. However, these results may reflect the fact that these drugs also produced the most robust reduction in MDMA-induced locomotion and that rearings are more likely to occur when an animal is not engaged in continuous forward locomotion. This conclusion is consistent with the observation that the direct DA receptor agonist apomorphine decreases investigatory behavior (Geyer *et al.*, 1987), but it contrasts with the observation that the DA-releasing agent (+)amphetamine dose not affect or increase investigatory behaviors (table 2). The differential sensitivity of crossings, holepokes and rearings to fluoxetine and PCPA suggests a dissociation

between the effects of (+)MDMA on locomotor activity and investigatory behavior.

Confirming previous studies (Gold *et al.*, 1988), the present data provide evidence that the spatial pattern of behavioral hyperactivity produced by MDMA differs qualitatively from the pattern elicited by a dose of amphetamine that produces similar amounts of activity. Although (+)amphetamine stimulates locomotion that is distributed throughout the entire testing chamber, (+)MDMA produced locomotion that was restricted largely to the periphery of the chamber. Furthermore, (+)amphetamine tends to increase investigatory holepokes and rearings (Geyer *et al.*, 1986; table 2), whereas (+)MDMA reduces both of these behaviors (Gold *et al.*, 1988; table 1). Thus, although (+)MDMA may resemble (+)amphetamine by virtue of its ability to increase the quantity of activity, the actual behavioral syndromes induced by each of these drugs appear to be quite distinct.

It is tempting to speculate that (+)MDMA and amphetamine exert stimulant-like effects *via* qualitatively different mechanisms. The present data support the conclusion that (+)MDMA-induced hyperactivity is dependent on drug-induced 5-HT release, raising the possibility that enhanced release of 5-HT is the direct cause of this behavioral activation. In contrast, amphetamine is believed to produce locomotor hyperactivity by increasing DA release in the mesolimbic DA pathway (Kelly *et al.*, 1975). Significant 5-HT release reportedly occurs only after doses of amphetamine greater than those usually used to evoke locomotor hyperactivity (Kuczenski, 1983). Although the failure of AMPT to block the effects of (+)MDMA in the present study demonstrates that direct release of DA by (+)MDMA is not necessary for the stimulant-like effects of (+)MDMA, an alternative explanation for the present observations is that (+)MDMA-induced activation of serotonergic systems indirectly increases DA release. There is evidence that 5-HT can affect the activity of dopaminergic neurons in the midbrain (Dray *et al.*, 1976), although 5-HT may primarily inhibit the firing of dopaminergic neurons (Ugedo *et al.*, 1989). In drug discrimination paradigms, however, MDMA does not substitute for amphetamine (Oberlander and Nichols, 1988) or for another psychostimulant putatively acting *via* dopaminergic stimulation, cocaine (Broadbent *et al.*, 1989). Thus, the qualitatively different syndromes elicited by (+)MDMA and (+)amphetamine may reflect the effects of central serotonergic and dopaminergic stimulation, respectively.

The present conclusion that 5-HT release by MDMA increases activity is paradoxical in view of previous observations that 5-HT release inhibits behavioral stimulation by catecholamines (Hollister *et al.*, 1976; Lucki and Harvey, 1979; Mabry and Campbell, 1973; Warbritton *et al.*, 1978). Similarly, both 5-HT_{1A} and 5-HT₂ agonists decrease rather than increase locomotor activity in the same paradigm used in the present studies of (+)MDMA (Mittman and Geyer, 1989; Wing *et al.*, 1990). However, the effects of MDMA may differ from the results of interventions using direct 5-HT receptor agonists or preloading with 5-HT precursors. Direct 5-HT agonists may stimulate receptors not normally exposed to endogenous 5-HT and also may differ from 5-HT in their potencies for activating the different 5-HT receptor subtypes (Peroutka and Snyder, 1979). Preloading with 5-HT precursors is also nonspecific inasmuch as pharmacological doses of these agents may result in secretion of 5-HT by nonserotonergic, aromatic amino acid

decarboxylase-containing neurons (Korf *et al.*, 1974). In contrast, MDMA releases endogenous 5-HT from presynaptic terminals, and thus the pattern of 5-HT receptor activation by MDMA probably closely resembles the normal pattern of serotonergic innervation.

Other treatments that increase the release of 5-HT and increase activity include administration of PCA (Frey and Magnussen, 1968) or of 5-hydroxytryptophan after peripheral decarboxylase inhibition (Schlosberg and Harvey, 1979). Some data also indicate that the hypermotility induced by PCA is dependent on 5-HT rather than catecholamine release (Lassen, 1974; Ogren and Johansson, 1985). In other behavioral paradigms, increased serotonergic activity does not appear to slow the habituation of rats to startling stimuli (Geyer and Tapson, 1988), and behavioral stimulation mediated by 5-HT release is consistent with electrophysiologic evidence indicating that firing of serotonergic neurons in the dorsal raphe nucleus is positively correlated with arousal (Trulsson and Jacobs, 1979). These latter studies thus provide support for the hypothesis that 5-HT release can be behaviorally activating.

In summary, the present study confirmed the behavioral potency of the 5-HT-releasing (+)isomer of MDMA for stimulating locomotion in rats. Preventing (+)MDMA-induced 5-HT releasing using 5-HT uptake inhibition or 5-HT synthesis inhibition attenuated this locomotor stimulation, suggesting that 5-HT release is necessary for the stimulant effects of MDMA. Finally, release of catecholamines appears to be less important in the behavioral effects of MDMA inasmuch as catecholamine synthesis inhibition did not attenuate the response (+)MDMA. These data indicate that MDMA and amphetamine produce hyperactivity by qualitatively different mechanisms, perhaps involving presynaptic release of 5-HT and DA, respectively.

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