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Amphetamine derivatives induce locomotor hyperactivity by acting as indirect serotonin agonists

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Abstract. Derivatives of amphetamine are potent releasers of both dopamine (DA) and serotonin (5–HT), but the relative contributions of DA and 5–HT release to the behavioral effects of these drugs have not been established. Previously, S-(+)3,4-methylenedioxymethamphetamine (S-(+)MDMA) was found to produce locomotor hyperactivity in rats which was dependent on 5–HT release. The present study found that MBDB (1.25, 2.5, 5.0 or 10.0 mg/kg), the alpha-ethyl derivative of MDMA that produces little or no direct DA release, also induced locomotor hyperactivity that lasted for greater than 60 min after the 5.0 and 10.0 mg/kg doses. MBDB produced spatial patterns of locomotor hyperactivity and suppression of exploratory activity (holepokes and rearings) very similar to the behavioral syndrome produced by MDMA. MBDB-induced hyperactivity was blocked by pretreatment with the selective 5–HT uptake inhibitor fluoxetine (2.5 or 10 mg/kg), suggesting that MBDB produced behavioral effects via uptake-carrier mediated release of 5–HT. Similarly, fluoxetine pretreatment blocked the locomotor hyperactivity produced by *S*-(+)3,4-methylenedioxyamphetamine (3.0 mg/kg) or *p*-chloroamphetamine (2.5 mg/kg), supporting a serotonergic basis for the action of these drugs. Tissue levels of 5–HT and its metabolite 5–HIAA were decreased 40 min after administration of *S*-(+)MDMA (3.0 mg/kg) or MBDB (5.0 mg/kg), and these decreases were prevented by fluoxetine pretreatment. *S*-(+)MDMA also produced a fluoxetine-sensitive increase of tissue DA levels, suggesting that 5–HT release may indirectly result in increased DA release, although MBDB did not significantly increase DA levels. These results point to a central role for 5–HT release in the stimulant-like behavioral effects of substituted derivatives of amphetamine.

Key words: Amphetamine – Methyenedioxyamphetamine – Methylenedioxymethamphetamine – MBDB – *p*-Chloroamphetamine – Fluoxetine – Locomotion – Serotonin – Rats

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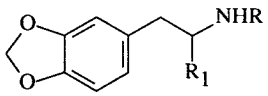
Considerable evidence supports the hypothesis that release of the neurotransmitter dopamine (DA) is responsible for the psychostimulant properties of amphetamine (Kelly et al. 1975). In contrast, substituted derivatives of amphetamine have novel neurochemical actions as well as varying potencies for DA release. For example, substitutions at the 2 and 5 positions of the phenethylamine moiety are associated with the hallucinogenic properties of the *R*-enantiomers of drugs such as DOB, DOET and DOM (Anderson et al. 1978), possibly by increasing the affinities of these substances for central serotonin (5–HT) receptors (Glennon et al. 1984). In contrast, phenethylamines with substitutions at the 3 and/or 4 positions have *S*-enantiomers that are potent 5–HT-releasing agents (Nichols et al. 1982; Johnson et al. 1986; Steele et al. 1987) but are weak direct receptor agonists (Lyon et al. 1986; Battaglia et al. 1988a). Interestingly, these latter drugs may not be hallucinogenic, but instead produce stimulant-like or mood-altering effects (Anderson et al. 1978). Because most of the substituted derivatives of amphetamine possess some DA-releasing properties, the respective contributions of DA or 5–HT release to the unique behavioral profile of the 3,4-substituted phenethylamines remains unclear.

Qualitative differences between the behavior induced by MDMA and that induced by amphetamine suggested that different neurochemical processes mediate the effects of these two drugs (Gold et al. 1988; Oberlender and Nichols 1988). In support of this hypothesis was the observation that fluoxetine, a selective 5–HT uptake inhibitor (Wong et al. 1975), attenuated MDMA-induced hyperactivity (Callaway et al. 1990). Because phenethylamine-induced release of monoamines has been shown to depend upon an intact monoamine uptake carrier (Fischer and Cho 1979; Raiteri et al. 1979) and 5–HT uptake inhibitors antagonize MDMA-induced 5–HT release in vitro (Hekmatpanah and Peroutka 1990), we concluded from these data that MDMA-induced hyperactivity in rats depends upon 5–HT release rather than DA release. Further supporting this conclusion, inhibition of 5–HT synthesis with *p*-chlorophenylala-



MDA R = H, $R_1 = CH_3$
MDMA R = CH_3, $R_1 = CH_3$
MBDB R = CH_3, $R_1 = CH_2CH_3$

Fig. 1. Molecular structures of MDA, MDMA and MBDB

nine, but not inhibition of catecholamine synthesis with alpha-methyl-*p*-tyrosine, attenuated MDMA-induced hyperactivity (Callaway et al. 1990).

In order to confirm that 5–HT release was responsible for the behavioral effects of MDMA, the present study was designed to determine whether the alpha-ethyl homolog of MDMA, N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB; Fig. 1), also produced fluoxetine-sensitive hyperactivity in rats. In contrast to MDMA, MBDB produces no direct DA release in vitro (Johnson et al. 1986). Fluoxetine pretreatment was also used to assess the role of 5–HT release in the hyperactivity produced by the N-desmethyl parent of MDMA, 3,4-methylenedioxyamphetamine (MDA) and by another 4-substituted phenethylamine, *p*-chloro-amphetamine (PCA). MDA and PCA resemble MDMA by virtue of being mixed DA and 5–HT releasing agents (Johnson et al. 1986; Hiramatsu and Cho 1990). Furthermore, the pattern of neurochemical changes induced by MDMA and MBDB was examined for consistency with the hypothesis that direct 5–HT release contributes to their behavioral effects.

Materials and methods

Animals. Male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) were housed two per cage in a temperature and humidity controlled vivarium under a 12 h:12 h reverse light-dark cycle (lights off: 0700 hours). Food and water were available ad libitum. Animals were allowed 10–14 days acclimation after arrival prior to any behavioral testing. Weights ranged from 350 to 420 g for behavioral studies, and from 175 to 200 g for biochemical studies.

Behavioral testing. The Behavioral Pattern Monitor (BPM) has been described previously (Flicker and Geyer 1982; Geyer et al. 1986). Briefly, the BPM consists of a 30.5 cm × 61.0 cm Plexiglas chamber through which a 4 × 8 grid of infrared photobeams is projected to allow localization of the animal in an X–Y plane. Three 2.5 cm holes are placed at equal intervals in each of the long walls and in the floor of the chamber, and a single hole is placed in one short wall. Photocells in these holes can detect investigatory nosepokes (holepokes). Animal rearings against the walls are detected by continuity between the smooth metal floor and a touchplate located 15.2 cm above the floor. The status of all photobeams and the touchplate are monitored continuously by computer and stored for subsequent off-line analysis.

Off-line analysis of the behavioral data was accomplished using programs written in C and executed on an IBM–PC compatible computer. Briefly, the raw data consisting of binary representations of photobeam status at particular clock times was converted into an ASCII file that contained a sequential list of four variables: the X and Y coordinates of the animal in the BPM, a flag signifying the occurrence of a holepoke or rearing and the duration spent at that particular coordinate or engaged in that particular behavior. This ASCII file was subsequently analyzed by other subroutines to produce the specific measures that are reported. Locomotor activity was quantified by the number of crossings between any of eight equal square regions within the BPM (crossings), as previously described (Geyer et al. 1986; Callaway et al. 1990). Data were further analyzed to calculate the number and durations of holepokes and rearings. Analysis of the spatial structure of rat locomotor paths was performed by calculating a descriptive statistic, d. The statistic d is based conceptually on fractal geometry and calculated using scaling arguments, as described in detail by Paulus and Geyer (1991). Changes in d reflect smoother (decreases in d) or rougher (increases in d) locomotor paths. Initially, data were examined in 10 min time resolution, analyses being conducted using one- or two-way ANOVA with time as a repeated measure (Dixon 1988). Post hoc comparisons of specific values were accomplished using Tukey's Studentized Range Method.

Animals were tested during the dark phase in darkness. For the dose-response study animals received a single injection of saline vehicle or MBDB (1.25, 2.5, 5.0 or 10.0 mg/kg, s.c), and 10 min later were placed into the BPM. For the interaction studies, animals received saline vehicle or fluoxetine (2.5 or 10.0 mg/kg, IP) 50 min before administration of MBDB (5.0 mg/kg, SC), *S*–MDA (3.0 mg/kg, SC) or PCA (2.5 mg/kg, SC). The saline/saline and fluoxetine/saline groups were shared for the interaction studies. Animals were placed into the BPM 10 min after administration of the test drug. Behavior was monitored for 60–120 min.

Biochemistry. Rats were injected with either saline vehicle or 10.0 mg/kg fluoxetine (IP) 50 min before administration of saline, *S*–MDMA (3.0 mg/kg, SC) or MBDB (5.0 mg/kg, SC). Animals were then sacrificed 40 min after administration of the test drug. The frontal cortex region was dissected from each animal and immediately frozen in liquid nitrogen. The tissue samples were stored at −70° C until assayed. Tissue samples were weighed and homogenized in 0.5 ml 0.4 N $HClO_4$ containing 0.5% Na_2EDTA, 0.1% $Na_2S_2O_5$ and 50 ng/ml N-acetyl-5–HT as an internal standard using a teflon pestle designed to accommodate 1.5 ml Eppendorf tubes. The samples were then centrifuged at 15000 *g* for 5 min with a table-top centrifuge and the supernatant assayed for norepinephrine (NE), DA and 5–HT using HPLC–EC techniques. The primary 5–HT metabolite 5-hydroxyindoleacetic acid (5–HIAA) as well as the DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were also assayed. A reverse-phase C18 analytical cartridge column (Brownlee Laboratories, Ann Arbor, MI) was eluted with a mobile phase containing 0.05 M NaH_2PO_4, 0.03 M citric acid, 0.1 mM Na_2EDTA, 0.025% sodium octyl sulfate and 25% methanol (pH = 2.75). The electrochemical detector (Model 400, EG & G Princeton Applied Research, Lawrenceville, NJ) utilized dual glassy carbon electrodes in series, with $E_1 = -200$ mV and $E_2 = 850$ mV. A flow rate of 1.2 ml/min gave the following retention times: NE, 3.7 min; DOPAC, 4.0 min; 5–HIAA, 5.0 min, N-acetyl-5–HT, 5.8 min; DA, 6.4 min; HVA, 7.9 min; and 5–HT, 12.2 min. The levels of monoamines and their metabolites were quantitated using the Dynamax HPLC manager software (Rainin, Woburn, MA) implemented on a Macintosh SE computer (Apple, Cupertino, CA). Statistical comparisons between different groups were performed using ANOVA followed by post hoc comparisons embodied in the Epistat software (Epistat services, Richardson, TX).

Drugs. NIDA provided *S*-(+)MDMA and *S*-(+)MDA. PCA was purchased from Sigma (St. Louis, MO). Fluoxetine was donated by Lilly (Indianapolis, IN). MBDB was synthesized as previously described (Nichols et al. 1986). All drugs were dissolved in saline and administered in a volume of 1 ml/kg, except for fluoxetine which was administered in a volume of 2 ml/kg.

Results

MBDB dose-response

MBDB produced a dose-dependent increase in locomotor activity as measured by crossings [$F(4,33) = 9.57$; $P < 0.0001$] (Fig. 2). Saline-treated animals were very active at the beginning of the session but became less active during habituation to the BPM, as indicated by a significant effect of time on crossings [$F(5,165) = 114.2$; $P < 0.0001$]. In contrast, MBDB-treated animals exhibited sustained levels of motor activity, resulting in a significant interaction between dose and time for crossings [$F(20,165) = 5.26$; $P < 0.0001$]. Figure 3 displays the computer reconstruction of the locomotor path produced during the first 30 min of the session by the animal that produced the median number of crossings after the 10.0 mg/kg dose of MBDB. After MBDB administration, animals produced relatively straighter locomotor paths. In some cases, this straightening of the locomotor path was manifested as continuous forward locomotion at the periphery of the chamber, similar to the rotation observed after MDMA (Gold et al. 1988). Unlike circling induced by direct receptor agonists such as apomorphine, rats receiving MBDB often changed the direction in which they circled the chamber. Confirming this visual impression that MBDB resulted in less varied locomotor paths, MBDB administration produced a dose-dependent reduction of the descriptive statistic d. Values of d (mean ± SEM) were 1.533 ± 0.018, 1.503 ± 0.010, 1.503 ± 0.019, 1.466 ± 0.030 and 1.389 ± 0.014 for the saline, 1.25, 2.5, 5.0 and 10.0 mg/kg MBDB groups, respectively [$F(4,33) = 7.50$, $P < 0.001$]. Individual comparisons revealed that d differed significantly from the saline group only for the 10.0 mg/kg MBDB group ($P < 0.01$).

The habituation of saline-treated animals to the BPM resulted in a progressive decrease of investigatory behaviors over time as reflected by a significant effect of time on holepokes [$F(5,165) = 4.43$; $P < 0.001$] and rearings [$F(5,165) = 27.64$; $P < 0.0001$] (Table 1). As previously reported for MDMA (Gold et al. 1988; Callaway et al. 1990) and in contrast to amphetamine (Geyer et al. 1987), MBDB dose-dependently decreased the absolute number of holepokes [$F(4,33) = 9.24$; $P < 0.0001$] and rearings [$F(4,33) = 16.59$; $P < 0.0001$]. Suppression of holepokes and rearings obscured the normal habituation to the BPM as reflected by a significant interaction between time and dose for holepokes [$F(20,165) = 3.33$; $P < 0.0001$] and rearings [$F(20,165) = 19.06$; $P < 0.0001$]. Because control animals produced few holepokes and rearings during the 30–60 min intervals, post hoc comparisons of holepokes or rearings between saline and MBDB groups were significant only during the 0–30 min time interval (Table 1).

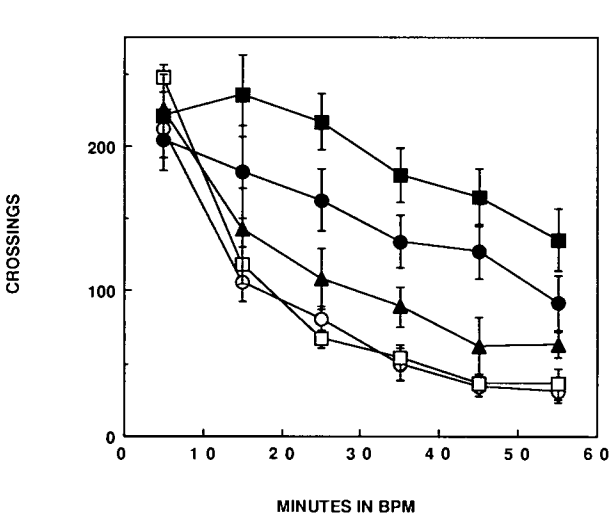


Fig. 2. Dose-dependent increase of locomotor activity by MBDB. Animals were injected with saline *(open circles)*, 1.25 *(open squares)*, 2.5 *(closed triangles)*, 5.0 *(closed circles)* or 10.0 mg/kg *(closed squares)* MBDB and 10 min later placed into the BPM. Behavior was recorded continuously for 60 min. Transitions between 8 equal square regions (crossings) per 10-min interval are indicated for times after the animal was placed into the BPM. Each point is the mean ± SEM for 7–8 animals

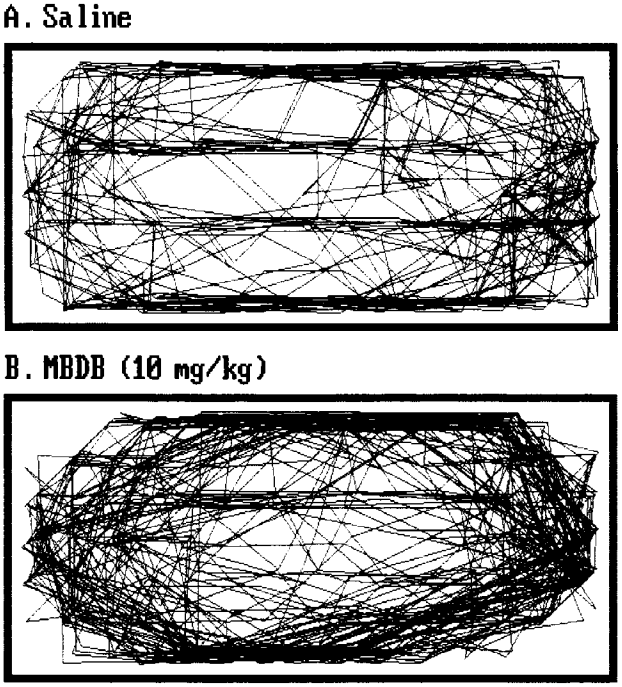


Fig. 3A, B. Locomotor path produced during 0–30 min interval after being placed into BPM by two different rats treated with saline (**A**) or 10 mg/kg MBDB (**B**). In contrast to the varied path with which the saline-treated animal explores the entire BPM, the MBDB-treated animal circles the periphery of the chamber and makes relatively fewer entries into the center

Effects of S-MDA and PCA

Based upon preliminary dose-response studies (M.A. Geyer, unpublished observations), doses of *S*-MDA (3.0 mg/kg) and PCA (2.5 mg/kg) were chosen for interaction studies because of their ability to produce hyperactivity without inducing myoclonus, forepaw treading, wet-dog shakes or other behaviors that charac-

Table 1. MBDB effects on investigatory behaviors

Dose (mg/kg) (n)	Saline (8)	1.25 (8)	2.5 (7)	5.0 (8)	10.0 (7)
Holepokes:					
0–30 min:	104.3 ± 9.8	86.1 ± 8.7	71.4 ± 13.9**	30.6 ± 4.9**	16.6 ± 1.9**
30–60 min:	63.0 ± 16.5	57.0 ± 9.4	78.4 ± 12.9	40.1 ± 7.7	26.9 ± 3.8
Rearings:					
0–30 min:	98.9 ± 9.2	94.0 ± 10.0	45.3 ± 12.6**	12.0 ± 4.8**	2.6 ± 1.0**
30–60 min:	27.0 ± 6.7	33.6 ± 5.8	43.9 ± 8.2	27.0 ± 6.4	10.1 ± 3.2

Animals were tested as in Fig. 2. Data are expressed as mean ± SEM
Statistical difference from saline group: $^{**}P < 0.01$

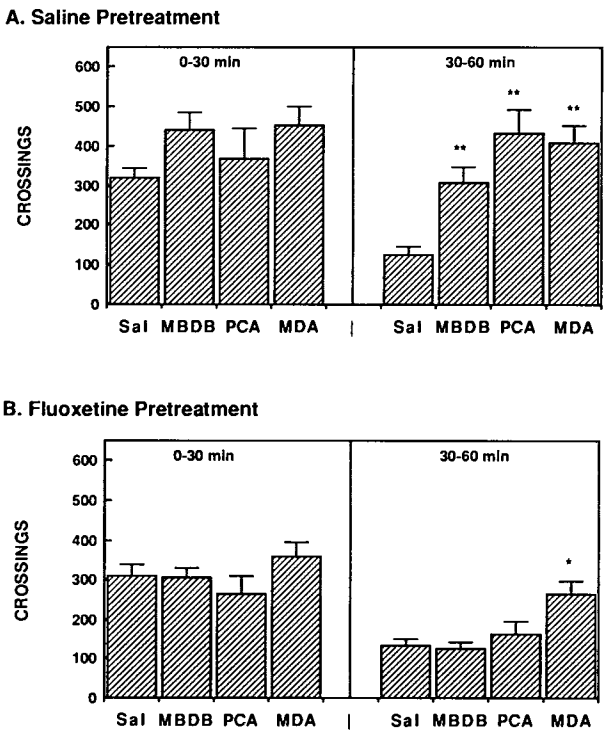


Fig. 4A, B. Interaction of fluoxetine with substituted amphetamine effects on locomotor activity. Saline or fluoxetine (10 mg/kg) was administered 50 min before saline, MBDB (5.0 mg/kg), PCA (2.5 mg/kg) or *S*–MDA (3.0 mg/kg). Animals were placed into the BPM 10 min after injection of the test drug, and behavior continuously recorded for 60 min. Crossings during the 0–30 min intervals and 30–60 min intervals after being placed into the BPM are indicated as mean ± SEM. MBDB, PCA and *S*–MDA increased crossings during the 30–60 min interval, and this increase was attenuated by pretreatment with fluoxetine ($^{*}P < 0.05$; $^{**}P < 0.01$)

terize the "serotonin syndrome" (Jacobs 1976). The 5.0 mg/kg dose of MBDB was chosen based upon the dose-response above, because this dose increased activity for the entire 60 min session. Significant drug effects on crossings (Fig. 4) confirm that locomotor activity was increased by MBDB [$F(1,34) = 6.59$; $P < 0.05$], PCA [$F(1,22) = 4.40$; $P < 0.05$] and *S*–MDA [$F(1,21) = 27.91$; $P < 0.0001$]. Furthermore, these drugs reduced both holepokes [MBDB: $F(1,34) = 24.86$; $P < 0.0001$; PCA: $F(1,22) = 17.83$; $P < 0.001$; *S*–MDA: $F(1,21) = 13.31$; $P < 0.01$] and rearings [MBDB: $F(1,34) = 24.23$; $P < 0.0001$; PCA: $F(1,22) = 41.66$; $P < 0.0001$; *S*–MDA: $F(1,21) = 26.18$; $P < 0.0001$] (Table 2). Inspection of plots of the locomotor path produced by rats receiving each of these drugs revealed that these drugs also decreased the proportion of excursions through the center of the BPM. Because rotation around the periphery of the chamber tends to produce a smoother locomotor path than does exploration of the entire chamber, this alteration in the spatial character of the locomotion is reflected by a reduction of the d statistic relative to saline controls ($d = 1.517 \pm 0.009$) by MBDB ($d = 1.480 \pm 0.039$), PCA ($d = 1.449 \pm 0.071$) and *S*–MDA ($d = 1.430 \pm 0.019$). While the mean values tend to confirm visual impressions about the spatial character of MBDB- and PCA-induced locomotor hyperactivity, post hoc comparisons revealed that the decrease in d was significant only after *S*–MDA administration ($P < 0.05$).

Fluoxetine pretreatment

Fluoxetine (10.0 mg/kg) by itself did not alter locomotor activity as measured by crossings (Fig. 4), but significantly reduced the MBDB- [Pretreatment × Drug Interaction: $F(1,34) = 7.17$; $P < 0.05$], PCA- [$F(1,22) = 5.30$; $P < 0.05$], and *S*–MDA-induced [$F(1,21) = 4.59$; $P < 0.05$] increases in crossings. Fluoxetine pretreatment by itself decreased both holepokes [$F(1,22) = 13.60$; $P < 0.01$] and rearings [$F(1,22) = 5.40$; $P < 0.05$]. Significant interactions between pretreatment and drug effects on holepokes and rearings were observed for MBDB [holepokes: $F(1,34) = 18.97$; $P < 0.001$; rearings: $F(1,34) = 16.92$; $P < 0.001$], PCA [holepokes: $F(1,22) = 9.36$; $P < 0.01$; rearings: $F(1,22) = 12.36$; $P < 0.01$] and *S*–MDA [holepokes: $F(1,21) = 7.61$; $P < 0.05$; rearings: $F(1,21) = 9.55$; $P < 0.01$]. In no case, however, did fluoxetine pretreatment appear to reverse the suppression of investigatory behaviors (holepokes and rearings) by the substituted amphetamines. These significant comparisons thus probably indicate that after the initial suppression of investigatory behaviors by fluoxetine, holepokes and rearings could not be further suppressed by the releasing drugs (Table 2). Fluoxetine also prevented the alteration in the spatial pattern of locomotor activity produced by MBDB, PCA and

Table 2. Effect of fluoxetine pretreatment on investigatory behaviors
A. Saline pretreatment

(n)	Saline (6)	MBDB (12)	PCA (7)	(+)MDA (6)
Holepokes:				
0–30 min:	118.5 ± 14.2	26.0 ± 5.2**	22.9 ± 4.7**	27.3 ± 5.3**
30–60 min:	81.3 ± 24.2	26.8 ± 5.3**	33.0 ± 5.5	41.5 ± 4.1*
Rearings:				
0–30 min:	81.5 ± 13.2	6.2 ± 3.0**	0.7 ± 0.6**	1.2 ± 0.6**
30–60 min:	23.0 ± 6.3	8.7 ± 3.1	1.3 ± 0.8**	15.5 ± 3.6

B. Fluoxetine pretreatment

(n)	Saline (7)	MBDB (13)	PCA (6)	(+)MDA (6)
Holepokes:				
0–30 min:	41.9 ± 7.6	31.5 ± 4.2	24.0 ± 2.9	25.7 ± 4.8
30–60 min:	24.7 ± 7.7	23.8 ± 4.1	19.3 ± 5.5	22.5 ± 6.2
Rearings:				
0–30 min:	31.4 ± 7.4	19.8 ± 4.3	5.2 ± 1.7	11.2 ± 2.8
30–60 min:	14.9 ± 2.8	16.1 ± 3.9	9.2 ± 4.0	12.0 ± 2.2

Animals were tested as in Fig. 4. Data are expressed as mean ± SEM
Statistical difference from corresponding pretreatment + saline group: $*P<0.05$; $**P<0.01$

S–MDA, as reflected by changes in the d statistic. By itself, fluoxetine did not affect d (1.523 ± 0.020). After fluoxetine pretreatment, further decreases in d were not produced by MBDB (d = 1.566 ± 0.013), PCA (d = 1.547 ± 0.023) or *S*–MDA (d = 1.575 ± 0.020). Only the *S*–MDA group produced a significant interaction between pretreatment and drug [$F(1,21) = 14.91$, $P<0.001$].

Because the significant reduction of holepokes and rearings by the 10.0 mg/kg dose of fluoxetine may have obscured any antagonism of the MBDB-induced reduction in investigatory behaviors, the response to 5.0 mg/kg MBDB was examined in a separate group of animals that had been pretreated with saline or 2.5 mg/kg fluoxetine (Fig. 5). In order to capture the full time-course of the response to MBDB, behavior was monitored in this study for 120 min. By itself, this dose of fluoxetine did not alter the number of crossings [$F(1,36) = 2.28$; $P>0.10$], the number of holepokes [$F(1,36) = 0.01$; $P>0.50$] or the number of rearings [$F(1,36) = 2.45$; $P>0.10$] during the session. Confirming the observations after 10.0 mg/kg fluoxetine, 2.5 mg/kg fluoxetine an-

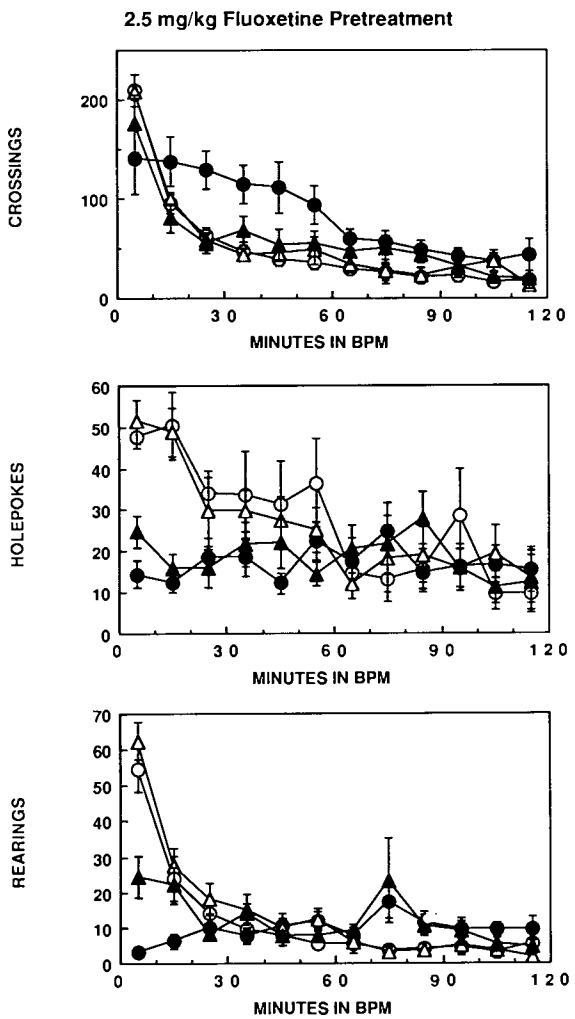


Fig. 5. Fluoxetine pretreatment reduces the locomotor hyperactivity produced by MBDB but does not reduce the MBDB-induced suppression of investigatory responding. Saline *(circles)* or 2.5 mg/kg fluoxetine *(triangles)* was administered 50 min before saline *(open symbols)* or 5.0 mg/kg MBDB *(closed symbols)*. Animals were placed into the BPM 10 min after injection of the test drug, and behavior continuously recorded for 120 min. Crossings, holepokes and rearings per 10-min interval are indicated for times after the animal was placed into the BPM. Each point is the mean ± SEM for 10 animals

Table 3. Effect of fluoxetine pretreatment on brain monoamine levels

Treatment	NE	DA	DOPAC	HVA	5-HT	5-HIAA
	(pg/mg wet weight)					
Saline/Saline	381.6 ± 27.2	69.6 ± 12.2	52.8 ± 5.0	85.3 ± 8.9	303.7 ± 9.8	234.6 ± 15.7
Saline/MDMA	402.5 ± 26.1	192.1 ± 30.4**	43.6 ± 5.3	86.5 ± 8.3	193.1 ± 15.4**	194.7 ± 8.8*
Saline/MBDB	363.4 ± 22.9	111.6 ± 19.5	53.3 ± 4.6	71.8 ± 8.6	167.0 ± 11.1**	161.3 ± 21.3**
Fluoxetine/Saline	366.3 ± 27.1	72.1 ± 7.0	48.3 ± 5.6	66.0 ± 5.9	280.9 ± 26.2	123.7 ± 4.7[a]
Fluoxetine/MDMA	369.3 ± 25.4	81.2 ± 10.2	38.3 ± 3.2	71.9 ± 4.4	295.8 ± 21.8	127.1 ± 7.1
Fluoxetine/MBDB	384.1 ± 28.2	91.2 ± 10.0	43.2 ± 4.0	72.8 ± 11.4	292.5 ± 12.3	125.7 ± 6.6

Groups of 6 rats were injected with either saline or 10 mg/kg fluoxetine, 50 min before injection of saline, *S*-MDMA (3.0 mg/kg) or MBDB (5.0 mg/kg). At 40 min following administration of the test drug, animals were sacrificed and monoamine levels assayed in frontal cortex. Data are expressed as mean ± SEM

Statistical difference from corresponding pretreatment + saline group: $^{*}P < 0.05$, $^{**}P < 0.01$

Statistical difference from saline + saline group: $^{a}P < 0.001$

tagonized the increase in crossings produced by MBDB during the first 60 min of the test session [Pretreatment × Drug × Time Interaction: $F(11,396) = 4.05$; $P < 0.05$]. However, 2.5 mg/kg fluoxetine did not significantly attenuate the decrease in holepokes or rearings produced by MBDB. Although the MBDB-induced decrease in rearings appeared to be partially reversed by 2.5 mg/kg fluoxetine during the first 20 min of the test session, this interaction was not statistically significant [Pretreatment × Drug × Time Interaction: $F(11,396) = 1.16$; $P > 0.25$]

Biochemistry

As seen in Table 3, *S*-MDMA or *RS*-MBDB produced a significant decrease in the levels of both 5-HT and its metabolite 5-HIAA at 40 min post-treatment. In addition, *S*-MDMA produced a significant increase in levels of DA, while MBDB produced a nonsignificant elevation of this transmitter. No significant changes in the DA metabolites, DOPAC or HVA, were observed.

Although 5-HT levels were unchanged after fluoxetine pretreatment, 5-HIAA levels were reduced. The decrease in the levels of 5-HT produced by *S*-MDMA or MBDB was prevented by pretreatment with the 5-HT uptake inhibitor fluoxetine. Furthermore, the increases in cortical DA levels after S-MDMA and MBDB were also attenuated by fluoxetine pretreatment, although fluoxetine by itself had no effect on DA levels.

Discussion

The primary finding of this study is that administration of amphetamine derivatives that are known to be potent 5-HT releasing agents increase locomotor activity in rats. Based on the behavioral profile assessed in the present study, MBDB, PCA and *S*-MDA resemble MDMA and not amphetamine. While the increase in the amount of locomotor activity produced by these drugs does not distinguish between amphetamine-like and MDMA-like actions, each of these drugs shared the ability of MDMA to decrease the frequency of investigatory behaviors (holepokes and rearings) (Gold et al. 1988, 1989; Callaway et al. 1990). Although amphetamine increases locomotor activity to a greater extent than investigatory behaviors, thereby decreasing the ratio between holepoke or rearing frequency and locomotor counts, amphetamine does not alter or increases the absolute frequency of investigatory behaviors (Geyer et al. 1986, 1987). Furthermore, locomotion after MBDB, *S*-MDA and PCA was often restricted to the periphery of the chamber, as has been reported for MDMA (Gold et al. 1988). As with *RS*-MDMA (Paulus and Geyer 1991), this spatial pattern of exploration was reflected by decreases in the descriptive statistic d. In contrast, previous work has indicated that amphetamine does not disrupt the spatial pattern of rat locomotor activity (Geyer et al. 1986), and at doses that do not induce focussed stereotypies, amphetamine also does not alter d (Paulus and Geyer 1991).

An alternative description for the present observations is that administration of MBDB and other 5-HT releasing agents retards the within-session habituation of motor activity. Consistent with this hypothesis, increases in 5-HT neurotransmission have been found to slow the habituation of the startle reflex in rats (Geyer and Tapson 1988). In the present study, a reduction in habituation does not explain the suppression of holepokes and rearings by serotonin-releasing drugs because the frequencies of these behaviors are greatest during the initial exposure of saline-treated animals to the BPM (Figs. 2, 4). Furthermore, previous studies have reported that MDMA increases locomotor activity in animals that are habituated to their surroundings (Gold et al. 1989; Spanos and Yamamoto 1989). Thus, the acute release of 5-HT appears to actually increase motor activity rather than specifically reducing habituation.

The fact that MBDB produced behavioral changes qualitatively similar to those produced by MDMA is consistent with the hypothesis that the behavioral effects of MDMA depend upon release of presynaptic stores of 5-HT (Callaway et al. 1990). While sharing the 5-HT releasing properties of MDMA, MBDB has been found to produce no direct DA release in vitro (Johnson et al. 1986), suggesting that direct DA release is not necessary for the behavioral effects that MBDB shares with

MDMA. Likewise, PCA and *S*–MDA produced behavioral profiles similar to those of MDMA and MBDB and not to that of amphetamine. As with MDMA, the hyperactivity induced by MBDB, MDA and PCA was sensitive to fluoxetine. By binding to the 5–HT uptake carrier, fluoxetine may interfere with the carrier-dependent release of the monoamine by the amphetamine derivatives (Fischer and Cho 1979; Raiteri et al. 1979; Hekmatpanah and Peroutka 1990). Therefore, these data support the hypothesis that 5–HT release contributes to the increase of locomotor activity produced by MBDB, PCA and MDA, although these studies do not explain the neurochemical mechanisms for the fluoxetine-insensitive suppression of investigatory-responding by 5–HT releasing drugs. Thus, it appears that despite the significant DA releasing properties that PCA and *S*–MDA share with amphetamine, the motor stimulant effects of these drugs result from the 5–HT releasing properties that they share with MDMA and MBDB.

Interestingly, the reduction of drug-induced locomotor hyperactivity by fluoxetine pretreatment was greatest for MBDB, which produces little or no direct DA release in vitro, and least for *S*–MDA, which has significant DA releasing properties (Johnson et al. 1986). PCA also produced a nonsignificant increase in the activity of fluoxetine-pretreated animals, as did *S*–MDMA in a previous study (Callaway et al. 1990). Because all of these drugs do possess some potency as DA releasing agents, the residual hyperactivity observed in fluoxetine-pretreated animals in response to *S*–MDA, PCA and *S*–MDMA might result from direct DA release. Alternatively, fluoxetine may have pharmacokinetic interactions with the substituted amphetamines that contribute to its differential interaction with each of these different phenethylamines (Ricaurte et al. 1983). Nevertheless, these behavioral data agree with suggestions that release of both DA and 5–HT contribute to the behavioral properties of substituted amphetamines (Yamamoto and Spanos 1988; Schechter 1989), and that the relative contributions of each amine may vary between drugs.

The alterations in tissue monoamine levels also point to a serotonergic mechanism of action for *S*–MDMA and MBDB. While several previous studies have examined the long-term toxicity produced by these drugs (Battaglia et al. 1988b), the present biochemical analyses were conducted at a time and dose corresponding to the acute behavioral stimulation produced by *S*–MDMA and MBDB (Fig. 2; Callaway et al. 1990). *S*–MDMA and MBDB reduced tissue levels of both 5–HT and 5–HIAA consistent with the ability of these drugs to release neuronal 5–HT as well as interfering with 5–HT reuptake (Johnson et al. 1986; Steele et al. 1987). The increase in tissue DA levels 40 min after *S*–MDMA was similar in magnitude to the increase of DA after higher doses of *RS*–MDMA at 3 h post-treatment (Johnson and Nichols 1989), and qualitatively similar to changes of neostriatal DA previously reported in response to MDMA (Stone et al. 1986; Schmidt et al. 1987). Similar changes in DA levels also have been observed after administration of amphetamine (Kuczenski 1977), methamphetamine (Koda and Gibb 1973; Kogan et al. 1976), and PCA (Leonard 1976). The fact that DA levels were not affected significantly by MBDB administration confirms in vitro data indicating that this compound is less potent than MDMA for increasing DA release (Johnson et al. 1986; Schmidt et al. 1987) and inhibiting synaptosomal DA reuptake (Steele et al. 1986).

The present data indicate that the acute 5–HT depletion induced by either MDMA or MBDB occurs via a fluoxetine-sensitive (i.e. 5–HT-uptake carrier-dependent) mechanism. Fluoxetine alone did not alter the tissue levels of 5–HT, suggesting that it lacks the direct 5–HT releasing properties of MDMA and MBDB. As in a previous report (Fuller et al. 1988), fluoxetine reduced tissue levels of 5–HIAA, consistent with its ability to prevent neuronal reuptake and subsequent deamination of 5–HT. After fluoxetine pretreatment, the MDMA and MBDB-induced decreases of 5–HT were prevented, and no further decreases in 5–HIAA levels were observed. To the extent that 5–HT depletion reflects 5–HT release, these results confirm that fluoxetine can prevent MDMA- and MBDB-induced 5–HT release.

Fluoxetine pretreatment also attenuated the increase in cortical DA after *S*–MDMA, suggesting that the increase in DA produced by *S*–MDMA administration depends upon 5–HT release. The fact that the selective 5–HT releasing agent MBDB did not alter DA levels significantly is inconsistent with this hypothesis, although the possibility of a dopaminergic action of MBDB at higher doses or different time-points cannot be excluded by the present biochemical data. It is unlikely that fluoxetine directly interfered with direct release of DA by *S*–MDMA, because of its low affinity for the DA uptake carrier relative to the 5–HT uptake carrier (Wong et al. 1975). Furthermore, the fact that DOPAC and HVA levels were unaltered by *S*–MDMA also suggested that the increased DA levels do not represent direct DA release, since levels of these metabolites typically are reduced by direct DA releasing agents (Kuczenski 1983). Other reports confirm that some effects of MDMA on dopaminergic systems may depend upon serotonergic mechanisms. For example, depletion or blockade of 5–HT can antagonize the effects of MDMA on neostriatal DA levels (Gazzara et al. 1989), DOPA accumulation (Nash et al. 1990) and nigrostriatal DA neuron firing (Kelland et al. 1989). In support of the hypothesis that MDMA-induced release of 5–HT increased dopaminergic transmission, infusion of 5–HT into the ventral tegmental area increases extracellular DA levels in the nucleus accumbens (Guan and McBride 1989). Alternatively, the increase in DA levels after *S*–MDMA (Table 3) may not reflect a primary action of the drug but may be an epiphenomenon of the drug-induced hyperactivity. By attenuating *S*–MDMA- or MBDB-induced hyperactivity, fluoxetine may thus indirectly block increases in DA levels. Nevertheless, these results point to close interactions between serotonergic and dopaminergic neurotransmission.

In conclusion, the present study indicates that the stimulant effects of *S*–MDMA on rat investigatory behavior are shared by MBDB, a congener of MDMA that lacks direct DA releasing properties. Moreover, *S*–MDA

and PCA, substituted derivatives of amphetamine with 5–HT-releasing properties, also produce effects qualitatively similar to those of MDMA. The behaviorally activating effects of each drug are sensitive to fluoxetine, suggesting that these effects are dependent upon uptake-carrier-mediated release of 5–HT. Supporting this conclusion, *S*–MDMA and MBDB produce changes in tissue monoamine levels that are consistent with 5–HT release, and these changes are blocked by fluoxetine pretreatment. To varying extents, direct and indirect DA release may also contribute to the behavioral effects of the substituted amphetamines. Fluoxetine-sensitive increases in DA levels after *S*–MDMA and MBDB suggest that drug-induced 5–HT release indirectly increases DA release. Although the locomotor-stimulant properties of MDMA and MBDB might be mediated in part via this indirect DA release, other data indicate that the activating effects of 5–HT receptor stimulation are not mediated via the DA system (Oberlander et al. 1986). Taken together, these results emphasize the importance of 5–HT release in the unique behavioral effects of these substituted phenethylamines.

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