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Suppression of behavioral activity by norfenfluramine and related drugs in rats is not mediated by serotonin release

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Abstract. Fenfluramine, a phenalkylamine with serotonin (5-HT) releasing properties, decreases motor activity in rats. The following studies assessed the contribution of 5-HT release to the behavioral effects of fenfluramine and norfenfluramine using a behavioral pattern monitor that simultaneously assesses locomotor and investigatory behavior. First, both fenfluramine and its active metabolite *d*-norfenfluramine dose-dependently reduced locomotor and investigatory activity. The norfenfluramine-induced reduction in activity was not antagonized by pretreatment with the 5-HT uptake inhibitor fluoxetine or the 5-HT synthesis inhibitor *p*-chlorophenylalanine, drugs that reduce drug-induced 5-HT release. Second, the *d*- and *l*-enantiomers of norfenfluramine were nearly equipotent at reducing behavioral activity, although *d*-norfenfluramine is more potent as a 5-HT releasing agent. Third, *p*-chloroamphetamine, a drug that shares the 5-HT releasing properties of fenfluramine produced locomotor hyperactivity in the same paradigm. Previous studies indicate that other 5-HT releasing phenalkylamines have behavioral effects resembling those of *p*-chloroamphetamine rather than those of fenfluramine. Finally, a structurally related drug, 4-methoxy-5-methyl-aminoindan (MMAI), produced dose-dependent reductions in behavioral activity that are similar to the effects of fenfluramine. The behavioral effects of MMAI were not antagonized by fluoxetine or by the 5-HT receptor antagonist methiothepin. These data suggest that the decrease in activity induced by fenfluramine, norfenfluramine and the related drug MMAI is not related to 5-HT release.

Key words: Serotonin – Fenfluramine – Locomotion – Behavior – Rats – *p*-Chloroamphetamine

Many behavioral phenomena are believed to involve functional alterations in the central nervous system neurotransmitter serotonin (5-HT). One approach toward elucidating the functional role of 5-HT is to examine the behavioral effects of drugs that influence serotonergic neurotransmission. For example, two halogenated derivatives of amphetamine, *p*-chloro-amphetamine (PCA) and (*m*-trifluoromethyl)-N-ethyl-amphetamine (fenfluramine) potently increase 5-HT release in vitro (Garrattini et al. 1975; Kannengiesser et al. 1976; McKenna et al. 1991) and in vivo (Sharp et al. 1986; Schwartz et al. 1989; Adell et al. 1989). This drug-induced increase in 5-HT release can be antagonized by inhibitors of the 5-HT reuptake carrier, suggesting that both fenfluramine and PCA release 5-HT via a carrier-mediated mechanism (Sanders-Bush and Steranka 1978; Hekmatpanah and Peroutka 1990). A similar mechanism is believed to mediate the catecholamine-releasing properties of the structural parent amphetamine (Fischer and Cho 1979; Raiteri et al. 1979).

Although many effects of fenfluramine and PCA have been studied, the serotonergic basis of these drug effects has been confirmed pharmacologically in only a few cases. For example, the appetite suppressant effect of fenfluramine can be antagonized by prior depletion of 5-HT (Kleven et al. 1988; Samanin et al. 1972). Similarly, both fenfluramine-induced increases in plasma prolactin levels (Van de Kar et al. 1985) and PCA-induced increases in plasma cortisol levels (Fuller and Snoddy 1980) can be antagonized by pretreatment with drugs that interfere with drug-induced 5-HT release. Relatively high doses of fenfluramine and PCA can induce abnormal motor behavior in many species that is also antagonized by prior 5-HT depletion (Trulson and Jacobs 1976). Thus, particular behavioral effects of fenfluramine and PCA are dependent on the 5-HT releasing properties of these drugs.

In contrast, other behavioral effects of fenfluramine administration have been attributed to 5-HT release without pharmacological testing. For example, one obvious behavioral effect of fenfluramine in humans is to produce lethargy or sedation (Gotestam and Gunne 1972; Griffith et al. 1975). This sedation is analogous to the fenfluramine-induced reduction in the quantity of motor activity in animals (Ziance et al. 1972; Lindquist and Gotestam 1977; Aulakh et al. 1988). These observations have been cited in support of the hypothesis that central 5-HT systems

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inhibit motor activity or are functionally antagonistic to central arousal mechanisms (Gerson and Baldessarini 1980; Soubrie 1986). However, this hypothesis is inconsistent with electrophysiological data indicating that serotonergic neuronal activity is positively correlated with both muscle activity (Steinfels et al. 1983) and behavioral arousal (McGinty and Harper 1976). Moreover, administration of PCA or other 5-HT-releasing derivatives of amphetamine increases motor activity in animals (Lassen 1974; Ogren and Johansson 1985; Callaway et al. 1991a). Other studies indicate that the drug-induced increase in motor activity is dependent on the 5-HT releasing properties of these drugs (Frey and Magnussen 1968; Ogren and Johannson 1985; Callaway et al. 1990, 1991a,b). Taken together, these data suggest that the reduction of motor activity by fenfluramine is unrelated to drug-induced 5-HT release.

In order to resolve the apparent discrepancy between the sedating effects of fenfluramine and the motor stimulant effects of other 5-HT releasing drugs, the following studies were designed to evaluate the contribution of 5-HT release to fenfluramine-induced reductions of spontaneous activity in rats. These studies employed a combined open-field and holeboard paradigm that has been useful for characterizing the unconditioned behavioral effects of several drug classes (Adams and Geyer 1985; Geyer et al. 1986). The first series of experiments characterizes the behavioral suppression induced by fenfluramine and its active metabolite norfenfluramine. The second series of experiments tests whether 5-HT release contributes to the behavioral response to the active *d*(+)-enantiomer of norfenfluramine by examining the effects of pretreatments with the 5-HT uptake inhibitor fluoxetine or the 5-HT synthesis inhibitor *p*-chlorophenylalanine (PCPA). Fluoxetine and PCPA have previously been found to antagonize the 5-HT releasing properties (Hekmatpanah and Peroutka 1990; McKenna et al. 1991) and behavioral effects of several amphetamine derivatives (Callaway et al. 1990, 1991a,b; Kehne et al. 1992) as well as the serotonergically mediated neuroendocrine effects of fenfluramine (Van de Kar et al. 1985) and PCA (Fuller and Snoddy 1980). For comparison, the behavioral effects of both PCA and an amphetamine derivative with fenfluramine-like pharmacological properties in vitro, 4-methoxy-5-methyl-aminoindan (MMAI) (Johnson et al. 1991), are presented.

Materials and methods

Animals. Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 300–400 g were housed two per cage under reverse light-dark cycle (lights off: 0700–1900) and constant ambient temperature (20 °C). At least 10–14 days acclimation to these housing conditions were allowed before the beginning of any experiments. Testing was performed in darkness during the dark phase of the light-dark cycle (between 0900 and 1400).

Behavioral measures. Behavioral measures were identical to those described previously (Callaway et al. 1990; 1991a). The Behavioral Pattern Monitor (BPM) is a 30.5 cm by 61.0 cm Plexiglas box with a smooth metal floor and walls extending 37.5 cm above the floor. A 4 × 8 grid of infrared photobeams projected through the BPM localizes the animal in an X-Y plane with a resolution of 3.8 cm. Three 2.5 cm holes are placed at equal intervals 2.5 cm above the floor in each long wall, and a single hole is centered in one end wall. Three holes are also equally spaced along the long axis of the floor. Infrared photobeams detect nosepokes into the holes (holepokes). A metal touchplate 15.2 cm above the floor monitors vertical motion (rearings).

The status of all photobeams and the rearing touchplate is monitored with time resolution of 55 ms by an IBM-PC compatible computer using customized interfacing hardware and software (San Diego Instruments, San Diego, CA). Horizontal locomotor activity is quantified by the number of transitions between eight equal sectors (15.2 cm × 15.2 cm) during any specified time interval ("crossings"). The frequency and duration of holepokes and rearings are also calculated for specific intervals.

The spatial character of locomotor activity is assessed visually using graphical reconstructions of the locomotor path followed by an animal during the session. Entries into the center region of the BPM were counted, and the ratio of center entries to crossings ("corrected center entries") were calculated as an index of thigmotaxis (Geyer et al. 1986). Corrected center entries are differentially affected by several drug classes (Adams and Geyer 1985; Geyer et al. 1987). In addition, a descriptive statistic, the spatial scaling exponent ("d"), is derived from the sequence of X-Y positions describing the locomotor path (Paulus and Geyer 1991). The d statistic is calculated by examining the path length through the BPM using several different spatial resolutions. The relationship between the measured path length and the spatial resolution can be described by an exponential function for which d is a coefficient of the spatial resolution. Thus, high values of d indicate locomotor paths that contain many local movements, where the measured path length decreases quickly with decreasing spatial resolution. Conversely, low values of d indicate locomotor paths that contain many distance-covering movements for which the measured path length decreases slowly with decreasing spatial resolution. Previously, the d statistic has proven useful for characterizing the behavioral effects of serotonergic drugs (Callaway et al. 1991a, b; Paulus and Geyer 1992).

Procedure. For dose-response studies, animals were brought to the testing area in darkness and allowed at least 60 min acclimation to the laboratory. Drugs were then administered by subcutaneous (SC) or intraperitoneal (IP) injection. At 10 min post-injection, the animals were placed into the BPM and behavior was monitored for 60 or 120 min. For interaction studies, the pretreatment drug or its vehicle was administered in the laboratory 30 min (methiothepin) or 50 min (fluoxetine) prior to the test drug. For the depletion study, PCPA or its vehicle was administered to each animal in its home cage 48 h prior to testing. The test drug was administered as before. Each animal was placed into the BPM at 10 min post-injection and behavior was monitored for 60 min.

Biochemistry. The effects of PCPA pretreatment on striatal tissue levels of dopamine (DA), homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), norepinephrine (NE), 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were assessed using HPLC with electrochemical detection. Immediately after the behavioral study, animals were decapitated and the brains quickly dissected into cold saline. The striatum was dissected by hand and stored at −70 °C until assayed. For HPLC analyses, mobile phase (0.08 M citric acid, 9% EtOH, 0.1 mM EDTA and 0.08 M octane sulfonate adjusted to pH 4.2) was delivered at a rate of 1.2 ml/min to a 250 mm × 4.6 mm ODS-C18 5 μm column maintained at 35 °C. Immediately prior to analysis, tissue was sonicated in mobile phase and centrifuged. A 10 μl aliquot of the filtered supernatant was injected into the HPLC. Amines were detected by a glassy carbon electrode maintained at +0.65 V relative to a Ag/AgCl reference electrode.

Statistics. Data were initially examined in 10 min epochs. For statistical comparisons, one-way or two-way analyses of variances were performed in which pretreatment, if any, and treatment were taken as independent factors and the value of each measure at multiple time-points was treated as a repeated measure (Dixon 1990).

Criterion for significance was set at $P < 0.05$. Greenhouse-Geisser and Hyunh-Feldt corrected probabilities were calculated when a significant interaction between time and another factor was observed in order to correct for correlations between repeated measures (Dixon 1990). If these corrected probabilities were also significant, only the uncorrected probability was reported. Where appropriate, data were then presented in 30- or 60-min epochs. Posthoc comparisons were performed using Tukey's Test with the criterion for significance set at $P < 0.05$ or $P < 0.01$. Biochemical data were analyzed by two-way analysis of variance with pretreatment and treatment as factors.

Drugs. *d*(+)-Norfenfluramine, *l*(−)-norfenfluramine and MMAI were synthesized in the laboratory of D.E.N. Fenfluramine, PCA HCl and PCPA methyl ester (Sigma, St Louis MO) were purchased. Fluoxetine HCl was a gift from Eli Lilly (Indianapolis, IN). All drugs were dissolved in saline and given in a volume of 1 ml/kg except for fluoxetine, which was administered in a volume of 2 ml/kg. Doses are based on salt weights except for PCPA for which the free base dose is given.

Results

Fenfluramine dose-response

Racemic fenfluramine (0.625, 1.25, 2.50 or 5.0 mg/kg, IP) administered 10 min prior to testing produced a dose-dependent reduction in locomotor activity relative to saline controls (Fig. 1), resulting in a significant drug effect on crossings ($F = 7.92$; $df = 4,38$; $P < 0.001$). Significant decreases in holepokes ($F = 10.42$; $df = 4,38$; $P < 0.0001$) and rearings ($F = 11.84$; $df = 4,38$; $P < 0.0001$) indicated a reduction in investigatory activity after fenfluramine (Table 1). After administration of fenfluramine, rats tended to remain motionless next to the walls of the BPM. This pattern of activity was reflected by a significant decrease in corrected center entries ($F = 3.54$; $df = 4,38$; $P < 0.05$) (Table 1). Possible changes in the spatial structure of locomotor paths were assessed after fenfluramine administration using the d statistic. In contrast to other 5-HT releasing drugs that produce robust decreases in this measure (Callaway et al. 1991a; Paulus and Geyer 1991), no significant change in d was observed after any dose of fenfluramine ($F = 2.09$; $df = 4,38$; N.S.) (Table 1).

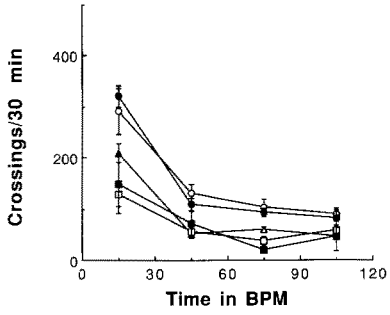

Fig. 1. Dose-dependent reduction of locomotor activity by fenfluramine. Saline or fenfluramine (0.625–5.0 mg/kg) was administered by IP injection 10 min prior to placing animals into the testing chamber. Behavior was monitored for 120 min. Each point represents the mean ± SEM number of crossings per 30 min interval for 8–10 animals. (–○–) Saline; (–●–) 0.625; (–△–) 1.25; (–□–) 2.50; (–■–) 5.00 mg/kg fenfluramine

Fenfluramine delayed injection

In the same series of experiments, a separate group of rats was treated with fenfluramine (2.50 mg/kg, IP) 40 min prior to testing. The behavioral effects of fenfluramine did not depend on preinjection interval as indicated by the lack of a significant difference between the 10-min and 40-min fenfluramine injections for crossings ($F = 0.08$; $df = 1,16$; N.S.) (data not shown), corrected center entries ($F = 0.39$; $df = 1,16$; N.S.), holepokes ($F = 1.75$; $df = 1,16$; N.S.), rearings ($F = 2.04$; $df = 1,16$; N.S.) or spatial d ($F = 3.29$; $df = 1,16$; N.S.) (Table 1).

d-Norfenfluramine dose-response

d-Norfenfluramine (0.625, 1.25 or 2.50 mg/kg, SC) administered 10 min prior to testing produced a dose-dependent reduction in crossings ($F = 17.91$; $df = 3,28$; $P < 0.0001$) (Fig. 2). Significant decreases in holepokes ($F = 68.06$; $df = 3,28$; $P < 0.0001$) and rearings ($F = 9.73$; $df = 3,28$; $P < 0.0001$) indicated a reduction in investigatory activity after *d*-norfenfluramine (Table 2). In contrast to the administration of fenfluramine, administration of *d*-norfenfluramine produced only a trend towards a decrease in

Table 1. Effects of fenfluramine on investigatory behaviors and spatial patterning of exploration

Treatment	(*n*)	Holepokes[a]	Rearings[a]	Corrected center entries[a]	Spatial D[b]
Saline	(8)	215.6 ± 34.1	136.4 ± 24.0	0.32 ± 0.05	1.59 ± 0.02
Fenfluramine					
0.625 mg/kg	(9)	198.1 ± 31.5	130.2 ± 17.3	0.36 ± 0.02	1.58 ± 0.04
1.25 mg/kg	(10)	89.2 ± 10.9**	75.0 ± 14.7	0.28 ± 0.03	1.59 ± 0.01
2.5 mg/kg	(9)	42.9 ± 5.6**	20.0 ± 4.1**	0.18 ± 0.04	1.61 ± 0.04
5.0 mg/kg	(7)	62.4 ± 33.0**	18.7 ± 16.8**	0.13 ± 0.06*	1.70 ± 0.06
Fenfluramine (delayed)					
2.5 mg/kg	(9)	73.0 ± 22.0**	46.7 ± 26.1	0.16 ± 0.15	1.71 ± 0.04

[a] Values are mean ± SEM for 0 to 120-min interval
[b] Values are mean ± SEM for 0 to 60-min interval
* Significantly different from Saline group, $P < 0.05$
** Significantly different from Saline group, $P < 0.01$

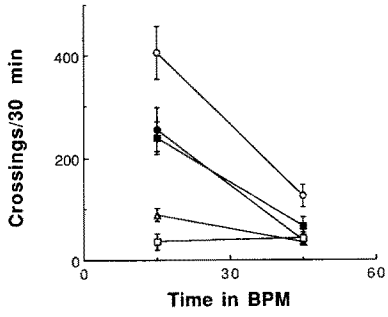

Fig. 2. Dose-dependent reduction of locomotor activity by *d*-norfenfluramine. Saline, *d*-norfenfluramine (0.625–2.50 mg/kg) or fenfluramine (5.0 mg/kg) was administered by SC injection 10 min prior to placing animals into the testing chamber. Behavior was monitored for 60 min. Each point represents the mean ± SEM number of crossings per 30-min interval for 7–10 animals. (-○-) Saline; (-●-) 0.625; (-△-) 1.25; (-□-) 2.50 mg/kg *d*-norfenfluramine (-■-) 2.5 mg/kg fenfluramine

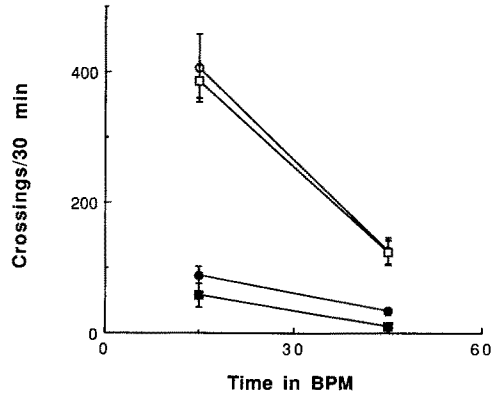

Fig. 3. Interaction of fluoxetine and *d*-norfenfluramine. Saline or fluoxetine (10.0 mg/kg) was administered by IP injection 60 min prior to testing. Saline or *d*-fenfluramine (1.25 mg/kg) was administered by SC injection 10 min prior to testing. Behavior was monitored for 60 min. Each point represents the mean ± SEM number of crossings per 30-min interval for 8–10 animals. (-○-) Saline/saline; (-●-) saline/norfenfluramine; (-□-) fluoxetine/saline; (-■-) fluoxetine/norfenfluramine

corrected center entries ($F=2.31$; $df=3,28$; $P<0.10$). A significant increase in the d statistic was observed after *d*-norfenfluramine ($F=7.64$; $df=3,28$; $P<0.001$), reflecting a tendency for *d*-norfenfluramine-treated animals to exhibit more local activity and less distance-covering activity than saline-treated animals (Table 2).

In order to confirm that the behavioral effects of fenfluramine were also evident after subcutaneous administration, a separate group of rats in the same study was treated with racemic fenfluramine (2.5 mg/kg, SC) 10 min prior to testing. This dose of fenfluramine reduced crossings ($F=5.19$; $df=1,15$; $P<0.05$) (data not shown), holepokes ($F=17.40$; $df=1,15$; $P<0.001$) and rearings ($F=5.45$; $df=1,15$; $P<0.05$) (Table 2). No significant fenfluramine effects on corrected center entries ($F=1.02$; $df=1,15$; N.S.) or spatial d ($F=0.00$; $df=1,15$; N.S.) were observed (Table 2).

d-Norfenfluramine fluoxetine interaction

In the same series of experiments, separate groups of animals received fluoxetine (10 mg/kg, IP) 50 min prior to administration of saline or *d*-norfenfluramine (1.25 mg/kg). No effect of fluoxetine on crossings ($F=0.65$; $df=1,30$; N.S.) and no interaction between fluoxetine and *d*-norfenfluramine for crossings ($F=0.12$; $df=1,30$; N.S.) could be detected (Fig. 3). Fluoxetine by itself produced a significant reduction in the number of holepokes during the 0–30 min time interval (Saline/Saline = 142.7 ± 13.0; Fluoxetine/Saline = 66.6 ± 13.9) ($F=22.17$; $df=1,30$; $P<0.0001$). This reduction obscured any further reduction of holepokes by *d*-norfenfluramine, as reflected by a significant interaction between fluoxetine and *d*-norfenfluramine for holepokes (Saline/Norfenfluramine = 17.5 ± 4.9; Fluoxetine/Norfenfluramine = 8.1 ± 2.3) ($F=13.39$; $df=1,30$; $P<0.001$). Rearings were not significantly altered by fluoxetine ($F=3.04$; $df=1,30$; N.S.), and fluoxetine did not alter the *d*-norfenfluramine-induced reduction of rearings ($F=2.75$; $df=1,30$; N.S.) (data not shown). Like *d*-norfenfluramine, fluoxetine did not significantly alter corrected center entries ($F=0.96$; $df=1,30$; N.S.) or spatial d ($F=0.07$; $df=1,30$; N.S.). No interaction between fluoxetine and *d*-norfenfluramine was observed for corrected center entries ($F=1.96$; $df=1,30$; N.S.) or spatial d ($F=2.16$; $df=1,30$; N.S.) (data not shown).

Table 2. Effects of *d*-norfenfluramine on investigatory behaviors and spatial patterning of exploration

Treatment	(*n*)	Holepokes[a]	Rearings[a]	Corrected center entries[a]	Spatial D[a]
Saline	(10)	214.1 ± 16.2	108.2 ± 14.7	0.31 ± 0.02	1.64 ± 0.02
d-Norfenfluramine					
0.625 mg/kg	(8)	72.4 ± 10.2**	40.8 ± 14.7*	0.21 ± 0.03	1.65 ± 0.01
1.25 mg/kg	(8)	24.4 ± 4.8**	4.1 ± 1.8**	0.36 ± 0.10	1.72 ± 0.03
2.5 mg/kg	(6)	11.8 ± 3.6**	0.2 ± 0.2**	0.24 ± 0.10	1.78 ± 0.03**
Fenfluramine					
2.5 mg/kg	(6)	27.7 ± 5.3**	0.2 ± 0.2**	0.24 ± 0.04	1.64 ± 0.02

[a] Values are mean ± SEM for 0 to 60-min interval
* Significantly different from Saline group, $P<0.05$
** Significantly different from Saline group, $P<0.01$

d-Norfenfluramine/PCPA interaction

Separate groups of rats were pretreated with saline or PCPA (200 mg/kg, IP) 72 h prior to testing. Saline or *d*-norfenfluramine (1.25 mg/kg, SC) was administered 10 min prior to testing. Crossings were reduced by both PCPA pretreatment ($F=33.13$; $df=1,36$; $P<0.0001$) and *d*-norfenfluramine treatment ($F=162.83$; $df=1,36$; $P<0.0001$) (Fig. 4). No significant interaction between PCPA and *d*-norfenfluramine for crossings was observed ($F=0.13$; $df=1,36$; N.S.), indicating that PCPA pretreatment did not antagonize the *d*-norfenfluramine-induced reduction of crossings. A significant Time × Pretreatment × Treatment interaction for crossings ($F=3.59$; $df=5,180$; $P<0.01$) reflects the fact that the near-maximal inhibition of locomotor activity in the PCPA/*d*-norfenfluramine group at later time-points prevented the effects of the two drugs from being strictly additive. PCPA pretreatment significantly reduced holepokes ($F=4.81$; $df=1,36$; $P<0.05$) and rearings ($F=11.14$; $df=1,36$; $P<0.01$) (data not shown). No interaction was observed between PCPA and *d*-norfenfluramine for holepokes ($F=1.45$; $df=1,36$; N.S.) or for rearings ($F=3.58$; $df=1,36$; N.S.). However, a significant Time × Pretreatment × Treatment interaction for rearings ($F=2.65$; $df=5,180$; $P<0.05$) indicates that the suppression of rearings by PCPA during the 30 to 60-min time interval (Saline/Saline = 28.6 ± 6.6; PCPA/saline = 9.7 ± 2.8) obscured any further reduction of rearings by *d*-norfenfluramine (Saline/Norfenfluramine = 0.5 ± 0.4; PCPA/norfenfluramine = 0.4 ± 0.3). No effect of PCPA on corrected center entries was observed in this study, but the marginal reduction of this measure by *d*-norfenfluramine did reach significance ($F=4.81$; $df=1,36$; $P<0.05$) (data not shown). The d statistic was slightly increased by PCPA (Saline/Saline = 1.62 ± 0.01; PCPA/saline = 1.64 ± 0.01) ($F=10.94$; $df=1,36$; $P<0.01$), and by the combination of PCPA and *d*-norfenfluramine (Saline/Norfenfluramine = 1.59 ± 0.02; PCPA/Norfenfluramine = 1.68 ± 0.02) ($F=5.14$; $df=1,36$; $P<0.05$).

Biochemical analyses indicated that PCPA pretreatment significantly reduced tissue levels of 5-HT ($F=34.51$; $df=1,36$; $P<0.0001$), 5-HIAA ($F=36.56$; $df=1,36$; $P<0.0001$) and HVA ($F=6.66$; $df=1,36$; $P<0.05$) (Table 3). No effect of PCPA on NE, DA or DOPAC levels was detected. In contrast, *d*-norfenfluramine-treated animals exhibited lower tissue levels of DOPAC ($F=4.15$; $df=1,36$; $P<0.05$) and HVA ($F=5.06$; $df=1,36$; $P<0.05$). No effect of *d*-norfenfluramine on 5-HT, 5-HIAA, NE, DA or DOPAC was observed. No significant interaction between PCPA and *d*-norfenfluramine was observed for any transmitter or metabolite.

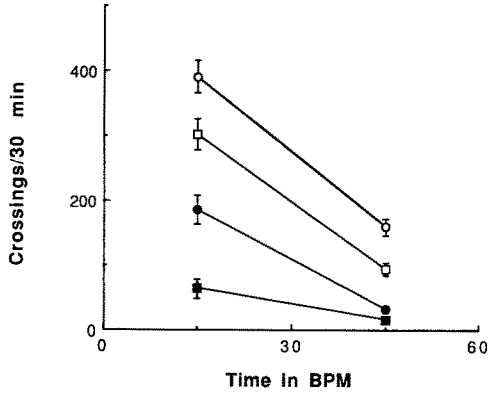

Fig. 4. Interaction of PCPA and *d*-norfenfluramine. Saline or PCPA (200 mg/kg) was administered by IP injection 72 h prior to testing. Saline or *d*-fenfluramine (1.25 mg/kg) was administered by SC injection 10 min prior to testing. Behavior was monitored for 60 min. Each point represents the mean ± SEM number of crossings per 30-min interval for 10 animals. (-○-) Saline/saline; (-●-) saline/norfenfluramine; (-□-) PCPA/saline; (-■-) CPA/norfenfluramine

l-Norfenfluramine single dose

Administration of *l*-norfenfluramine (1.25 mg/kg, SC) 10 min prior to testing reduced crossings during the 0 to 30-min interval (saline = 405.7 ± 22.1; *l*-norfenfluramine = 230.2 ± 29.7) as reflected by a significant Time × Treatment interaction for crossings ($F=6.57$; $df=11,198$; $P<0.0001$). *l*-Norfenfluramine administration significantly reduced holepokes ($F=6.69$; $df=1,18$; $P<0.05$), particularly during the 0 to 30-min interval (Saline = 137.2 ± 14.9; *l*-Norfenfluramine = 47.6 ± 5.5), but not rearings ($F=2.06$; $df=1,18$; N.S.). A significant Time × Treatment interaction for rearings ($F=11.28$; $df=11,198$; $P<0.0001$) reflects the fact that *l*-norfenfluramine did reduce rearings during the 0 to 30-min interval

Table 3. Effects of *p*-chlorophenylalanine (PCPA) and *d*-norfenfluramine on striatal monoamine levels in rats

Monoamine	Concentrations (pmol/mg tissue)[a]			
Pretreatment:	Saline	Saline	PCPA	PCPA
Treatment:	Saline	Norfenfluramine	Saline	Norfenfluramine
(*n*)	(10)	(10)	(10)	(10)
5-HT	4.1 ± 0.5	4.1 ± 1.0	0.8 ± 0.3**	0.4 ± 0.1**
5-HIAA	9.5 ± 1.0	9.6 ± 1.3	3.9 ± 0.5**	3.9 ± 0.7**
NE	0.7 ± 0.2	1.3 ± 0.5	0.9 ± 0.1	0.7 ± 0.2
DA	120.6 ± 9.1	121.4 ± 12.0	132.9 ± 6.9	105.3 ± 9.0
DOPAC	12.1 ± 1.2	11.5 ± 1.2	12.5 ± 0.7	8.9 ± 0.9
HVA	9.0 ± 0.8	8.0 ± 2.9	7.8 ± 0.6	5.3 ± 0.7**

[a] Values are mean ± SEM

** Significantly different from saline pretreatment group, $P<0.01$

(Saline = 99.8 ± 12.1; *l*-Norfenfluramine = 27.1 ± 14.3). *l*-Norfenfluramine produced no significant effect on corrected center entries ($F = 0.04$; $df = 1,18$; N.S.) or spatial d ($F = 0.72$; $df = 1,18$; N.S.) (data not shown).

PCA dose-response

After administration of PCA (0.625–5.0 mg/kg, IP), animals exhibited increased locomotor activity, resulting in a significant increase in crossings, particularly at the 2.5 mg/kg dose ($F = 4.68$; $df = 4,44$; $P < 0.01$) (Fig. 5). PCA produced significant reductions in investigatory holepokes ($F = 5.55$; $df = 4,44$; $P < 0.01$) and rearings ($F = 23.20$; $df = 4,44$; $P < 0.0001$) (Table 4). The spatial pattern of activity was altered by PCA, as reflected by significant reductions in corrected center entries ($F = 4.53$; $df = 4,44$; $P < 0.01$) and spatial d ($F = 5.65$; $df = 4,44$; $P < 0.001$) (Table 4). Interestingly, spatial d was significantly decreased only after the 2.5 mg/kg dose of PCA, because a subset of animals exhibited very local patterns of activity after the 5.0 mg/kg dose. This increase in local activity at higher doses of PCA corresponds to the emergence of stereotyped motor activity associated with the "serotonin syndrome" that has been reported to occur in this dose range (Trulson and Jacobs 1976). These patterns of activity resemble the effects of other 5-HT releasing derivatives of amphetamine (Callaway et al. 1991a, b).

MMAI dose-response

Administration of MMAI (0.3, 1.0, 3.0 or 10.0 mg/kg, SC) produced a dose-dependent decrease in locomotor activity as reflected by a significant drug effect on crossings ($F = 5.59$; $df = 4,35$; $P < 0.01$) (Fig. 6). Like fenfluramine and norfenfluramine, MMAI also reduced holepokes ($F = 12.80$; $df = 4,35$; $P < 0.0001$) and rearings ($F = 4.04$; $df = 4,35$; $P < 0.01$) (Table 5). MMAI-treated animals tended to remain near the walls of the BPM, resulting in a significant decrease in corrected center entries ($F = 8.19$; $df = 4,35$; $P < 0.001$) (Table 5). The average spatial structure of locomotion as reflected by the d statistic was altered only at the highest dose of MMAI ($F = 3.83$; $df = 4,35$; $P < 0.05$) (Table 5). After this dose, locomotion was dramatically suppressed, resulting in a relatively greater contribution of local movements to overall activity, and a consequent reduction in spatial d.

MMAI, fluoxetine and methiothepin interaction

In a separate study, animals were pretreated with saline (SC or IP, 30 or 50 min), fluoxetine (10.0 mg/kg, IP, 50 min) or methiothepin (0.1 mg/kg, SC, 30 min) prior to the administration of saline or MMAI (3.0 mg/kg, SC). Pretreatment with fluoxetine did not prevent the suppression of locomotor activity by MMAI ($F = 0.16$; $df = 1,34$;

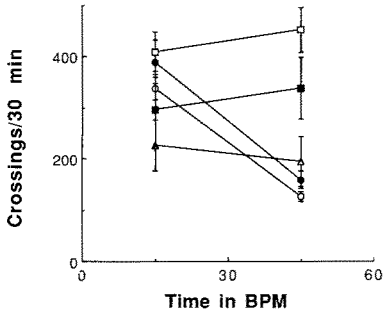

Fig. 5. Stimulation of locomotor activity by PCA. Saline or PCA (0.625–5.0 mg/kg) was administered by IP injection 10 min prior to placing animals into the testing chamber. Behavior was monitored for 60 min. Each point represents the mean ± SEM number of crossings per 30-min interval for 9–10 animals. (-○-) Saline; (-●-) 0.625; (-△-) 1.25; (-□-) 2.50; (-■-) 5.00 mg/kg PCA

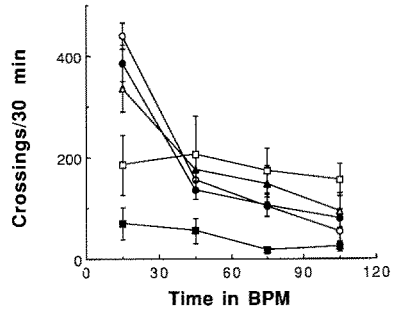

Fig. 6. Dose-dependent reduction of locomotor activity by MMAI. Saline or MMAI (0.3–10.0 mg/kg) was administered by SC injection 10 min prior to placing animals into the testing chamber. Behavior was monitored for 120 min. Each point represents the mean ± SEM number of crossings per 30-min interval for 8 animals. (-○-) Saline; (-●-) 0.30; (-△-) 1.0; (-□-) 3.0; (-■-) 10.0 mg/kg MMAI

Table 4. Effects of *p*-chloro-amphetamine (PCA) on investigatory behaviors and spatial patterning of exploration

Treatment	(*n*)	Holepokes[a]	Rearings[a]	Corrected center entries[a]	Spatial D[a]
Saline	(10)	143.0 ± 19.2	131.3 ± 11.8	0.28 ± 0.03	1.65 ± 0.01
PCA					
0.625 mg/kg	(9)	95.3 ± 15.0	106.7 ± 19.4	0.21 ± 0.03	1.60 ± 0.01
1.25 mg/kg	(10)	37.1 ± 5.5**	28.3 ± 9.0**	0.11 ± 0.08	1.67 ± 0.04
2.5 mg/kg	(10)	71.9 ± 12.2*	18.4 ± 10.6**	0.24 ± 0.04	1.45 ± 0.03**
5.0 mg/kg	(10)	87.9 ± 24.0	7.3 ± 5.0**	0.35 ± 0.08	1.57 ± 0.06

[a] Values are mean ± SEM for 0 to 60-min interval
* Significantly different from Saline group, $P < 0.05$
** Significantly different from Saline group, $P < 0.01$

Table 5. Effects of 4-methoxy-5-methyl-aminoindan (MMAI) on investigatory behaviors and spatial patterning of exploration

Treatment	(*n*)	Holepokes[a]	Rearings[a]	Corrected center entries[a]	Spatial D[b]
Saline	(8)	329.9 ± 18.1	98.4 ± 28.4	0.30 ± 0.04	1.64 ± 0.01
MMAI					
0.3 mg/kg	(8)	299.4 ± 42.1	153.5 ± 37.4	0.25 ± 0.03	1.62 ± 0.01
1.0 mg/kg	(8)	282.4 ± 62.3	92.1 ± 36.3	0.19 ± 0.04	1.61 ± 0.03
3.0 mg/kg	(8)	122.3 ± 21.9**	63.9 ± 14.6	0.10 ± 0.04**	1.65 ± 0.07
10.0 mg/kg	(8)	28.9 ± 13.8**	2.8 ± 2.8	0.04 ± 0.03**	1.83 ± 0.07

[a] Values are mean ± SEM for 0 to 120-min interval
[b] Values are mean ± SEM for 0 to 60-min interval
* Significantly different from Saline group, $P<0.05$
** Significantly different from Saline group, $P<0.01$

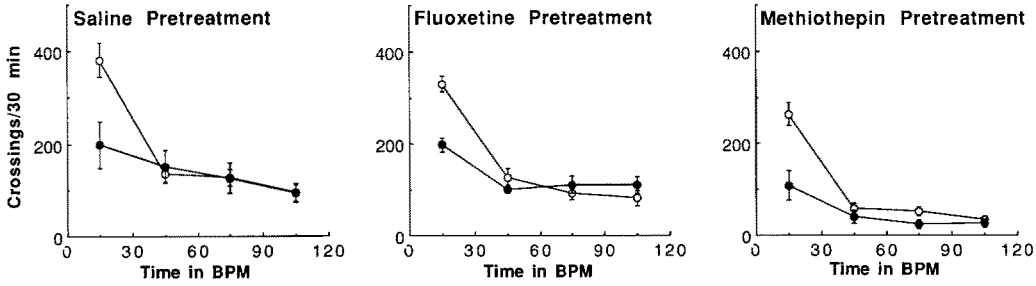

Fig. 7. Interaction of fluoxetine, methiothepin and MMAI. Saline or fluoxetine (10.0 mg/kg) was administered by IP injection 60 min prior to testing. Methiothepin (0.1 mg/kg) was administered by SC injection 30 min prior to testing. Saline (–○–) or MMAI (–●–) (3.0 mg/kg) was administered by SC injection 10 min prior to placing animals into the testing chamber. Behavior was monitored for 120 min. Each point represents the mean ± SEM number of crossings per 30-min interval for 9–11 animals

N.S.) (Fig. 7). However, significant Time × Pretreatment × Treatment interactions for holepokes ($F=2.02$; $df=11,374$; $P<0.05$) and rearings ($F=4.00$; $df=11,374$; $P<0.0001$) indicate that the initial suppression of holepokes and rearings by MMAI during the 0 to 30-min interval was partially antagonized by fluoxetine pretreatment (Holepokes: Saline/Saline = 165.9 ± 15.7; Fluoxetine/Saline = 118.7 ± 19.3; Saline/MMAI = 15.8 ± 4.8; Fluoxetine/MMAI = 54.4 ± 6.9. Rearings: Saline/Saline = 82.9 ± 13.5; Fluoxetine/Saline = 56.1 ± 12.6; Saline/MMAI = 9.2 ± 6.8; Fluoxetine/MMAI = 39.5 ± 12.3.). Although fluoxetine pretreatment increased the number of corrected center entries ($F=12.89$; $df=1,34$; $P<0.01$), fluoxetine did not antagonize the reduction in corrected center entries produced by MMAI (data not shown). This dose of MMAI did not alter spatial d (Saline/Saline = 1.66 ± 0.01; Saline/MMAI = 1.65 ± 0.06) ($F=0.27$; $df=1,34$; N.S.). No interaction between the effects of fluoxetine and MMAI was observed for spatial d ($F=0.01$; $df=1,34$; N.S.) (data not shown).

Pretreatment with methiothepin reduced crossings in both control and MMAI-treated animals ($F=22.45$; $df=1,36$; $P<0.0001$) (Fig. 7). However, the absence of any significant interaction between methiothepin and MMAI on crossings ($F=0.07$; $df=1,36$; N.S.) indicates that the reduction in activity induced by methiothepin was additive with the reduction produced by MMAI. Methiothepin reduced both holepokes ($F=20.52$; $df=1,36$; $P<0.0001$) and rearings ($F=9.93$; $df=1,36$; $P<0.01$) (data not shown). This effect of methiothepin partially obscured the further reduction of these measures by MMAI during the 30 to 60-min interval (Holepokes: Saline/Saline = 119.0 ± 17.4; Methiothepin/Saline = 26.4 ± 9.8; Saline/MMAI = 30.8 ± 8.8; Methiothepin/MMAI = 12.9 ± 6.8. Rearings: Saline/Saline = 25.3 ± 4.4; Methiothepin/Saline = 3.1 ± 1.4; Saline/MMAI = 7.9 ± 3.9; Methiothepin/MMAI = 4.2 ± 2.9), resulting in a significant Pretreatment × Treatment interaction for holepokes ($F=5.98$; $df=1,36$; $P<0.05$) and a significant Time × Pretreatment × Treatment interaction for rearings ($F=1.86$; $df=11,396$; $P<0.05$). Methiothepin did not antagonize the reduction in corrected center entries induced by MMAI ($F=0.06$; $df=1,36$; N.S.) (data not shown). In contrast, methiothepin increased local activity, resulting in an increase in spatial d (Saline/Saline = 1.66 ± 0.01; Methiothepin/Saline = 1.71 ± 0.01) ($F=6.33$; $df=1,36$; $P<0.05$). The absence of any significant interaction between MMAI and methiothepin for spatial d ($F=1.57$; $df=1,36$; N.S.) indicates that the marginal effect on spatial d produced by MMAI was additive with the increase produced by methiothepin (Saline/MMAI = 1.65 ± 0.06; Methiothepin/MMAI = 1.78 ± 0.03).

Discussion

The primary finding of this study was that the reduction of rat behavioral activity by fenfluramine or its active metabolite norfenfluramine was not related to 5-HT release.

First, norfenfluramine-induced suppression of motor activity was not stereoselective, unlike norfenfluramine-induced 5-HT release (Mennini et al. 1985). Second, the drug-induced reduction in activity was not antagonized by pretreatments that prevent drug-induced 5-HT release. Finally, a similar dissociation of 5-HT release and behavioral suppression was exhibited by MMAI, a drug with fenfluramine-like behavioral effects and in vitro pharmacology.

The present data confirm previous reports that fenfluramine administered systemically to rats decreases spontaneous motor activity (Ziance et al. 1972; Lindquist and Gotestam 1977; Aulakh et al. 1988). During the initial presentation to the BPM, rats treated with saline explored the chamber resulting in an increased number of crossings, investigatory holepokes and rearings. Each of these dependent variables was decreased by fenfluramine in a dose-dependent manner. The fenfluramine-induced reduction in corrected center entries corroborates the observation that fenfluramine-treated animals typically made a few excursions through the BPM at the start of the session and then remained quietly next to the wall for most of the remainder of the session.

Both fenfluramine and its metabolites produce similar suppression of activity. Moreover, the same suppression of activity was observed when fenfluramine was administered 10 min or 40 min prior to placing the rat into the BPM. The relative proportion of the primary fenfluramine metabolite norfenfluramine would be expected to be increased with longer preinjection times (Mennini et al. 1985). Therefore, the absence of any difference between the two groups suggests that fenfluramine and norfenfluramine produce similar effects on rat motor activity. The dose-dependent reduction of locomotor activity, holepokes and rearings by *d*-norfenfluramine confirms this hypothesis. However, *l*-norfenfluramine in a dose of 1.25 mg/kg was intermediate in potency between 0.625 mg/kg and 1.25 mg/kg of *d*-norfenfluramine at reducing behavioral activity, although the *l*-isomer of norfenfluramine is approximately two-fold less potent at releasing 5-HT than the *d*-isomer (Mennini et al. 1985). Thus, the absence of clear-cut stereoisomerism in the behavioral effects of norfenfluramine fails to support the hypothesis that drug-induced 5-HT release mediates norfenfluramine-induced suppression of behavioral activity.

These data are inconsistent with the hypothesis that drug-induced 5-HT release reduces motor activity (Gerson and Baldessarini 1980; Soubrie 1986). First, the behaviorally suppressive effects of fenfluramine contrast with the locomotor activating effects of PCA, MDMA and a variety of other 5-HT releasing drugs (Callaway et al. 1990, 1991a, b; Paulus and Geyer 1992). Similarly, PCA and MDMA, but not fenfluramine, produce increases in acoustic and tactile startle responses that are prevented by fluoxetine or by neurotoxin-induced depletions of central 5-HT (Kehne et al. 1992). Second, fluoxetine pretreatment did not prevent *d*-norfenfluramine-induced reductions in motor activity, suggesting that *d*-norfenfluramine did not reduce activity via uptake-carrier-dependent 5-HT release. The fluoxetine pretreatment used in the present study has previously been shown to antagonize behavioral (Callaway et al. 1990, 1991a; Kehne et al. 1992) and neuroendocrine effects (Fuller and Snoddy 1980; Nash et al. 1988) induced by other 5-HT releasing agents. Third, PCPA pretreatment produced a selective depletion of brain 5-HT without altering tissue NE or DA levels, but did not antagonize the *d*-norfenfluramine-induced reductions in motor activity. These data indicate that release of 5-HT via fluoxetine-insensitive mechanisms did not contribute to the *d*-norfenfluramine-induced suppression of activity. The absence of any measurable *d*-norfenfluramine effect on striatal tissue levels of monoamines at this dose and time-point is consistent with previous reports (Invernizzi et al. 1986).

Like fenfluramine, MMAI has biochemical effects in vitro that predict its action as an amphetamine-like 5-HT releasing agent (Johnson et al. 1991). The present studies indicated that the behavioral effects of MMAI resemble those of fenfluramine, consisting primarily of a reduction in rat motor activity. The decrease in behavioral activity by MMAI also was not antagonized by pretreatment with fluoxetine or with the mixed 5-HT_1 and 5-HT_2 receptor antagonist methiothepin. The fluoxetine dose and methiothepin dose used in the present study were effective at reducing hyperactivity induced by *S*-3,4-methylenedioxymethamphetamine (*S*-MDMA) in the same paradigm (Callaway et al. 1990, 1992), as well as the neuroendocrine and anorectic effects of fenfluramine in other paradigms (Van de Kar et al. 1985; Neill and Cooper 1989). These results indicate that increased 5-HT release does not contribute to the drug-induced suppression of motor activity by MMAI, and are consistent with the other data indicating that release of endogenous 5-HT does not mediate the behavioral suppression induced by norfenfluramine.

Recognizing that the sedating effects of fenfluramine are not serotonergically mediated prompts re-evaluation of the hypothesis that 5-HT release is associated with a suppression of activity (Gerson and Baldessarini 1980; Soubrie 1986). In addition, other observations that served as the basis for this hypothesis have been reinterpreted. Increased spontaneous activity in 5-HT-depleted animals, for example, has been found to be dependent upon illumination levels and environmental novelty (Hole et al. 1976; Lorens et al. 1976), suggesting that the apparent excitatory effects of 5-HT depletion in fact result from the inability of 5-HT-depleted animals to engage in normal sleep (Jacobs et al. 1972). As another example, the hyperactivity observed after electrolytic lesions of the raphe nuclei is not observed after specific neurochemical lesions that produce equivalent depletions of forebrain 5-HT (Lorens 1978). Recent observations of behavioral activation after administration of many 5-HT releasing drugs suggest that 5-HT release is in fact positively correlated with motor activity (Callaway et al. 1990, 1991a). However, the multiplicity of 5-HT receptor subtypes and potential sites of action discourage formation of any hypothesis that postulates a single function for 5-HT.

Although the present studies rule out 5-HT release as a possible mechanism, the pharmacological basis for suppression of rat exploratory behavior by fenfluramine, norfenfluramine and MMAI remains unclear. One possibility is suggested by biochemical studies in which fenfluramine produces neuroleptic-like actions (Bettini et al. 1987).

Both fenfluramine and norfenfluramine can antagonize behavioral stimulation induced by amphetamine or apomorphine (Bendotti et al. 1980). However, fenfluramine does not displace (^{3}H)-DA or (^{3}H)-haloperidol from brain tissue homogenates, indicating that neuroleptic-like effects of fenfluramine do not result from direct interactions with DA receptors (Burt et al. 1976), prompting the hypothesis that these drugs functionally antagonize dopaminergic neurotransmission presynaptically (Bendotti et al. 1980). Pharmacological studies to determine the contribution of 5-HT release to the neuroleptic-like actions of fenfluramine have not been performed, although some studies indicate that central serotonergic systems can modulate drug-induced catalepsy (Kostowski et al. 1972). However, if the neuroleptic-like properties of fenfluramine are independent of 5-HT release, these properties may account for the nonserotonergic inhibition of spontaneous motor activity observed in the present studies.

Nonserotonergic mechanisms may contribute to the suppression of rat exploratory behavior by other derivatives of amphetamine. For example, in contrast to the increases in locomotion, the suppression of holepokes and rearings produced by PCA in the present study is also insensitive to fluoxetine pretreatment (Callaway et al. 1991a). In addition, *S*-MDMA decreases both holepokes and rearings in the same paradigm used in the present study. These effects are not antagonized by fluoxetine, PCPA or receptor antagonist pretreatments that block *S*-MDMA-induced hyperactivity (Callaway et al. 1990, 1992). Similar fluoxetine-insensitive suppression of investigatory behaviors occurs after administration of *S*-3,4-(methylenedioxy)-amphetamine and the more selective 5-HT releasing agent N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (Callaway et al. 1991a). Moreover, a close congener of MMAI, 5,6-(methylenedioxy)-2-aminoindan produces a brief suppression of locomotor activity as well as investigatory behaviors. These suppressive effects are unaffected by a fluoxetine pretreatment that blocks the subsequent locomotor hyperactivity in the same animals (Callaway et al. 1991b). It is tempting to speculate that the nonserotonergic pharmacological properties that mediate the inhibition of motor activity after fenfluramine, norfenfluramine and MMAI in the present study also contribute to the behavioral suppression produced by other amphetamine derivatives.

In conclusion, the present studies confirm that fenfluramine and the related amphetamine-derivative MMAI reduce spontaneous behavioral activity in rats. This suppression of motor activity by fenfluramine is insensitive to pretreatments that prevent drug-induced 5-HT release and contrasts with the behavioral activation produced by other 5-HT releasing amphetamine derivatives. Therefore, these data indicate that in addition to acting as 5-HT releasing agents, norfenfluramine and MMAI reduce behavioral activity via a nonserotonergic mechanism. This result may be relevant to studies of serotonergic function in humans, where the sedating effects of fenfluramine have been taken as evidence that 5-HT release inhibits behavioral activity (Gotestam and Gunne 1972; Griffith et al. 1975). More recent studies have focussed on possible mood elevation by fenfluramine (Ward et al. 1985; Lichtenberg et al. 1992), in part because the mood-altering effects of other 5-HT releasing drugs such as MDMA are well known (Nichols and Oberlender 1990). These divergent conclusions illustrate the need for further studies to evaluate the pharmacological basis for the diverse clinical effects of fenfluramine and related drugs.

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