0022-3565/92/2631-0318$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

Vol. 263, No.
Printed in U.S.

Tolerance and Cross-Tolerance to the Activating Effects of 3,4-Methylenedioxymethamphetamine and a 5-Hydroxytryptamine$_{1B}$ Agonist[1]

CLIFTON W. CALLAWAY and MARK A. GEYER
Department of Psychiatry, School of Medicine, University of California San Diego, La Jolla, California
Accepted for publication June 3, 1992

ABSTRACT

Previous studies indicate that 3,4-methylenedioxymethamphetamine (MDMA) produces locomotor hyperactivity in rats by increasing the presynaptic release of serotonin (5-HT). Interactions between MDMA and 5-HT receptor antagonists suggest that the activating effects of MDMA are mediated *via* 5-HT$_1$-like receptors. In order to assess the contribution of particular 5-HT receptor subtypes to the behavioral effects of MDMA, the present studies examined the development of tolerance and cross-tolerance to the behavioral effects of MDMA and selective 5-HT receptor agonists. In the first study, rats were pretreated with saline, a 5-HT$_{1A}$ receptor agonist (8-hydroxy-2-(di-*n*-propylamino)tetralin, 1.0 mg/kg), a 5-HT$_{1B}$ receptor agonist [5-methoyx-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole butane dioate (RU24969), 2.5 mg/kg] or a 5-HT$_{1C}$/5-HT$_2$ receptor agonist (2,5-dimethoxy-4-iodoamphetamine, 1.0 mg/kg) twice daily for 3 days. The behavioral response to *S*-MDMA (3.0 mg/kg) was assessed 36 hr after the last pretreatment injection. Pretreatment with RU24969 antagonized the activating effects of *S*-MDMA. In contrast, pretreatment with 2,5-dimethoxy-4-iodoamphetamine did not alter the response to *S*-MDMA, and pretreatment with 8-hydroxy-2-(di-*n*-propylamino)tetralin reduced the activity of both control and *S*-MDMA-treated animals. In the second study, rats were pretreated with saline or *RS*-MDMA (10 mg/kg), twice daily for 4 days. The behavioral response to saline, *S*-MDMA (3.0 mg/kg), RU24969 (2.5 mg/kg) or *S*-amphetamine (1.0 mg/kg) was assessed 36 hr after the last pretreatment injection. Pretreatment with *RS*-MDMA antagonized the activating effects of *S*-MDMA and RU24969, but potentiated the activating effect of S-amphetamine. The antagonistic effect of *RS*-MDMA pretreatment on the behavioral response to *S*-MDMA and RU24969 was not evident when animals were retested 21 days later. Parallel biochemical studies indicate that the *RS*-MDMA pretreatment regimen induced a lasting depletion of forebrain 5-HT. Thus, the observation of acute reciprocal cross-tolerance between the activating effects of MDMA and the RU24969 suggests that the behavioral effects of these drugs are mediated by the same receptors. These results are consistent with the hypothesis that release of endogenous 5-HT increases locomotor activity *via* stimulation of 5-HT$_{1B}$ receptors.

Previous studies have suggested that pharmacological manipulations that increase the release of endogenous 5-HT are behaviorally activating in rats. In particular, substituted phenethylamines related to amphetamine are potent 5-HT-releasing agents as well as being catecholamine-releasing agents (Nichols *et al.*, 1986). One drug of this class, MDMA, produces locomotor hyperactivity in rats that is qualitatively different from the hyperactivity produced by amphetamine (Gold *et al.*, 1988; Spanos and Yamamoto, 1989). Pretreatments designed to prevent the release of presynaptic 5-HT by MDMA prevent the locomotor-activating effects of this drug (Callaway *et al.*, 1990). Other drugs that share the 5-HT-releasing properties of MDMA and that cause little or no catecholamine release also produce MDMA-like patterns of hyperactivity in rats (Callaway *et al.*, 1991). These data thus indicate that MDMA-induced hyperactivity results from the release of 5-HT.

Multiple subtypes of 5-HT receptors have been described, including 5-HT$_1$, 5-HT$_2$ and 5-HT$_3$ subtypes (Bradley *et al.*, 1986). Previous studies found that 5-HT$_1$ receptor antagonists, but not 5-HT$_2$ receptor antagonists, effectively block the activating effects of MDMA (Callaway *et al.*, 1992). In rat brain, 5-HT$_1$ receptors have been subclassified into 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1C}$ subtypes (Pazos *et al.*, 1984; Pedigo *et al.*, 1981).

Received for publication August 15, 1991.
[1] This work was supported by National Institute on Drug Abuse Award DA02925, National Institute of Mental Health Research Scientist Award MH00188 (M.A.G.) and National Institute of Health Medical Scientist Training Program M07198 (C.W.C.).

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); MDMA, 3,4-methylenedioxymethamphetamine; RU24969, 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole butane dioate; BPM, Behavioral Pattern Monitor; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; DOI, 2,5-dimethoxy-4-iodoamphetamine; DA, dopamine; HVA, homovanillic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; NE, norepinephrine; 5-HIAA, 5-hydroxyindoleacetic acid; HPLC, high-pressure liquid chromatography; LSD, lysergic acid diethylamide.

Unfortunately, antagonists that are selective for these various 5-HT receptor subtypes are either unavailable or poorly characterized. Although the behavioral effects of agonists with widely different selectivities for the various 5-HT receptor subtypes have been examined, locomotor activation is observed only after administration of agonists with affinity for 5-HT_1 receptors (Evenden and Angeby-Moller, 1990; Oberlander *et al.*, 1987; Tricklebank *et al.*, 1986). In the same paradigm in which MDMA and related drugs have been found to produce hyperactivity, 5-HT_{1A} (Mittman and Geyer, 1989) and 5-HT_{1C}/5-HT_2 agonists (Wing *et al.*, 1990) consistently reduce activity. Only the 5-HT_{1B} receptor agonist RU24969 has been found to produce hyperactivity that is qualitatively similar to the activity induced by 5-HT-releasing agents (Oberlander *et al.*, 1987; Rempel *et al.*, in press). These observations prompted the hypothesis that the locomotor-activating effects of 5-HT-releasing drugs such as MDMA are mediated by activation of 5-HT_{1B} receptors. The present study therefore used three agonists having relatively specific actions at 5-HT_{1A}, 5-HT_{1B} or 5-HT_{1C}/5-HT_2 receptors in order to assess the contribution of these 5-HT receptor subtypes to the behavioral effects of MDMA.

Many neurotransmitter receptors undergo adaptive changes of number or sensitivity in response to prolonged overstimulation. In particular, both 5-HT_1 and 5-HT_2 receptors have been found to down-regulate or desensitize during treatments that increase 5-HT availability (Savage *et al.*, 1980; Peroutka and Snyder, 1980). The present series of experiments used repeated administrations of selective 5-HT agonists to induce functional subsensitivity of particular 5-HT receptor subtypes and examined the effects of these pretreatments on the subsequent behavioral response to MDMA. The hypothesis that the activating effects of MDMA are mediated *via* 5-HT_{1B} receptors predicts that the response to MDMA will be reduced after 5-HT_{1B} agonist administration, but not after treatment with 5-HT_{1A} or 5-HT_2 receptor agonists. Conversely, the behavioral effects of a 5-HT_{1B} receptor agonist were assessed in animals made tolerant to the activating effects of MDMA by repeated administration of MDMA. The finding of specific cross-tolerance between *S*-MDMA and a 5-HT_{1B} receptor agonist strongly supports the hypothesis that 5-HT_{1B} receptors participate in the behavioral effects of *S*-MDMA.

Methods

Animals. Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 290 to 380 g at the start of experiments were housed two per cage under reverse light-dark cycle (lights off: 7:00 A.M. to 7:00 P.M.) with controlled ambient temperature (20°C). At least 10 to 14 days were allowed for acclimation to these housing conditions before the beginning of any experiments. All testing was performed in darkness during the dark phase of the light-dark cycle (between 9:00 A.M. and 2:00 P.M.).

Behavioral measures. Behavioral measures were obtained using methods identical with those described previously (Callaway *et al.*, 1990, 1991). The BPM is a 30.5-cm by 61.0-cm Plexiglas box with a smooth metal floor. A 4 × 8 grid of infrared photobeams is projected through the BPM to allow localization of 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 projected across each hole monitor animal investigation of the holes (hole-pokes). The walls of the box extend 37.5 cm above the floor. Electrical continuity is established when the animal simultaneously touches the floor and a metal touch-plate 15.2 cm above the floor, thereby monitoring vertical motion (rearings).

The status of all photobeams and the rearing touch-plate is monitored continuously by an IBM-PC-compatible computer using customized interfacing hardware and software (San Diego Instruments, San Diego, CA). All changes are stored along with their durations as sequential binary representations of photobeam and touch-plate status, allowing subsequent detailed reconstruction of the spatial and temporal sequences of behavioral events (Geyer *et al.*, 1986). Data analysis began by reducing the binary data into ASCII files. These files contain sequential lists of the X–Y position of the animal, flags indicating the occurrence of hole-pokes or rearings, and the length of time at a particular position or engaged in particular behaviors. Summary data for particular time intervals are calculated from these ASCII files. 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 hole-pokes and rearings are also calculated for specific intervals. These summary data can be read directly by statistical analysis software (Dixon, 1990).

The spatial character of locomotor activity is assessed visually using graphical reconstructions of the locomotor path followed by an animal during the session. Center, wall and corner regions within the BPM were defined as in previous studies in order to assess the spatial distribution of activity (Geyer *et al.*, 1986). 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). Based conceptually on fractal geometry, the *d* statistic is calculated by examining the path length through the BPM using several different spatial resolutions. Using scaling arguments, the rate at which the path length decreases as spatial resolution decreases is fitted to an exponential function for which "*d*" is a coefficient of the spatial resolution. Thus, high values of *d* indicate rougher locomotor paths, where the measured path length decreases quickly with decreasing spatial resolution, while low values of *d* indicate smoother locomotor paths for which the measured path length decreases slowly with decreasing spatial resolution.

Procedure. The first study examined the effect of subchronic pretreatment with selective 5-HT receptor agonists on the subsequent response to *S*-MDMA. Either saline, the 5-HT_{1A} agonist 8-OH-DPAT (1.0 mg/kg), the 5-HT_{1B} agonist RU24969 (2.5 mg/kg) or the 5-HT_{1C}/5-HT_2 agonist DOI (1.0 mg/kg) was given by s.c. injection at 12-hr intervals for 3 days. Between 35 and 37 hr after the final pretreatment injection, animals were brought from their home cages and allowed to acclimate to the laboratory for 1 hr. Each animal was placed into the BPM 10 min after being injected with saline or *S*-MDMA (3.0 mg/kg, s.c.), and behavior was monitored for 120 min. In order to assess tolerance to the locomotor-activating effects of RU24969, the saline-pretreated/saline-challenged and RU24969-pretreated/saline-challenged animals remained in the laboratory for 1 to 2 hr after testing in the BPM. RU24969 (2.5 mg/kg) was given to each animal by s.c. injection. At 10 min postinjection, the animals were then placed into the BPM and behavior was monitored for an additional 120 min.

The second study assessed the influence of MDMA pretreatment on the response to *S*-MDMA or RU24969. In order to identify whether MDMA pretreatment influenced the response to *S*-MDMA and RU24969 *via* nonspecific effects on activity levels, animals were also challenged with a dose of *S*-amphetamine that was approximately equipotent with *S*-MDMA for increasing locomotor activity. Previous studies have indicated that the behavioral stimulation produced by *S*-MDMA and amphetamine are mediated *via* different pharmacological mechanisms (Callaway *et al.*, 1990). Rats were pretreated with saline or *RS*-MDMA (10 mg/kg, s.c.) at 12-hr intervals for 4 days. Between 35 and 37 hr after the final pretreatment injection, animals were brought from their home cages and allowed to acclimate to the laboratory for 1 hr. Each animal was placed into the BPM 10 min after s.c. administration of saline, *S*-MDMA (3.0 mg/kg), RU24969 (2.5 mg/kg) or *S*-amphetamine (0.75 mg/kg), and behavior was monitored for 120 min. After testing, animals were returned to their home cages. In order

to dissociate short-term and persistent effects of MDMA-pretreatment, animals were brought to the laboratory again 21 days after the first testing. After being allowed to acclimate for 1 hr, each animal was given saline, *S*-MDMA (3.0 mg/kg) or RU24969 (0.625, 1.25 or 2.5 mg/kg) by s.c. injection. To control for any interaction between the drugs administered during the first and second test sessions, groups were balanced with respect to the treatment history of the animals. At 10 min postinjection, each animal was placed into the BPM and behavior was monitored for 60 min.

Biochemistry. The effects of repeated MDMA administration on regional brain levels of DA, HVA, DOPAC, NE, 5-HT and 5-HIAA were assessed in a separate group of animals. Each rat was pretreated with saline or MDMA according to the same regimen used for the behavioral studies. At 36 days after the last MDMA injection, animals were decapitated and brains quickly dissected into cold saline. Hippocampal formations and caudates were dissected by hand and stored at −70°C until assayed by HPLC with electrochemical detection. 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 (Waters) maintained at 35°C. Immediately before 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 +0.7 V relative to a Ag/AgCl reference electrode, and concentrations were estimated from peak areas using a Waters Maxima 820 data station.

Statistics. Data were initially examined in 10-min epochs. For statistical comparisons, a two-way mixed-design analysis of variance was performed in which pretreatment and treatment were treated as independent factors and the value of each measure at multiple timepoints was treated as a repeated measure (Dixon, 1990). Criterion for significance was set at $P < .05$. In order to correct for correlations between repeated measures, Greenhouse-Geisser and Hyunh-Feldt corrected probabilities were calculated when a significant interaction between time and another factor was observed. 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. *Post hoc* comparisons were performed using Tukey's studentized range method with the criterion for significance set at $P < .05$ or $P < .01$. Biochemical data were analyzed by one-way analysis of variance with pretreatment as the single factor.

Drugs. The National Institute on Drug Abuse provided *RS*-(±)-MDMA and *S*-(+)-MDMA. RU24969 was a gift of Roussel-Uclaf (Romainville, France). DOI and 8-OH-DPAT were purchased from Research Biochemicals Inc. (Natick, MA). *S*-(+)-amphetamine was purchased from Sigma (St. Louis, MO). All drugs were dissolved in saline and given in a volume of 1 ml/kg. Doses are based on salt weights except for *S*-amphetamine, for which the free base dose is given. Dose-response analyses have been presented previously in this paradigm for *RS*-MDMA (Gold *et al.*, 1988), *S*-MDMA (Callaway *et al.*, 1990), *S*-amphetamine (Geyer *et al.*, 1987), DOI (Wing *et al.*, 1990) and 8-OH-DPAT (Mittman and Geyer, 1989). A complete dose-response analysis for RU24969 is the subject of a separate report (Rempel *et al.*, in press).

Results

Behavioral effects of *S*-MDMA. The chronic agonist study confirmed the primary effects of *S*-MDMA in this paradigm. Briefly, *S*-MDMA produced a significant increase in locomotor activity as indicated by crossings ($F = 80.57$; dF = 1,52; $P < .0001$) (figs. 1 and 2). The spatial pattern of locomotor activity exhibited by *S*-MDMA-treated rats was significantly different from the pattern of activity exhibited by control animals as assessed by the spatial scaling exponent (d) ($F = 15.21$; dF = 1,52; $P < .001$) (table 1). This *S*-MDMA-induced decrease in the d statistic corresponds to a relative increase in the frequency of distance-covering movements and a concomitant decrease in the frequency of more localized mov
S-MDMA-treated animals produced fewer investigatory
iors, including hole-pokes (time × drug interaction: F =
dF = 11,572; $P < .0001$) (table 1). In addition, a sig
interaction between drug and time for rearings ($F = 3$
= 11,572; $P < .0001$) indicates that *S*-MDMA proc
biphasic effect on rearings. Relative to control anim
frequency of rearings was decreased during the initial
of the test session and increased between 30 and 90 m
the test session (table 1).

Acute effects of repeated agonist pretreatmen
treatment with RU24969 antagonized the increase in loc
activity produced by *S*-MDMA (pretreatment × tre
interaction: $F = 4.42$; dF = 1,28; $P < .05$) (figs. 1 and
spatial pattern of control or drug-induced locomotor
was not altered by RU24969 pretreatment as indicated
d statistic. After RU24969 pretreatment, both control an
treated animals exhibited fewer hole-pokes ($F = 29.01$
1,28; $P < .0001$) and rearings ($F = 8.98$; dF = 1,28; P
(table 1). A significant interaction between time, pretre
and treatment for rearings ($F = 4.88$; dF = 11,308; P <
indicates that RU24969 pretreatment reduced the *S*-M
induced increase of rearings during the 30- to 90-min i
(table 1). An apparently significant interaction betwee
pretreatment and treatment for hole-pokes ($F = 1.87$
11,308; $P < .05$) was not significant when Greenhouse-(
($P = .09$) or Hyunh-Feldt corrections were used (P
When rats from the saline/saline and RU24969/saline
were challenged with RU24969, RU24969 pretreatment
icantly reduced the locomotor-activating effects of RU24
= 121.06; dF = 1,14; $P < .0001$) (fig. 3) but did not alt
influence of RU24969 on any other behavioral measure.

Pretreatment with 8-OH-DPAT reduced the locomot
tivity of both saline-treated and *S*-MDMA-treated anim
= 7.81; dF = 1,26; $P < .01$) (figs. 1 and 2). No significant
of 8-OH-DPAT pretreatment and no significant inter
between 8-OH-DPAT pretreatment and *S*-MDMA on th
tial pattern of locomotor activity was evident from the d
tic. Similarly, 8-OH-DPAT pretreatment reduced hole-
($F = 10.96$; dF = 1,26; $P < .01$) without antagonizing the f
reduction of this measure by *S*-MDMA (table 1). 8-OH-I
did not alter the number of rearings exhibited by c
animals but did antagonize the increase in rearings prc
by *S*-MDMA during the second hour of testing (pretrea
× treatment × time interaction: $F = 3.30$; dF = 11,286
.001) (table 1).

Pretreatment with DOI did not alter the quantity or s
character of locomotor activity exhibited by saline-treat
S-MDMA-treated animals (figs. 1 and 2). DOI pretreat
slightly reduced the number of hole-pokes exhibited by c
animals (pretreatment × time: $F = 2.29$; dF = 11,286; P <
but did not antagonize the further reduction of this meas
S-MDMA (table 1). DOI reduced the number of rearing
hibited by control animals ($F = 6.34$; dF = 1,26; $P < .05$
antagonized the increase in rearings produced by *S*-M
during the second hour of testing (pretreatment × treatm
time interaction: $F = 3.34$; dF = 11,286; $P < .001$) (table

Acute effects of MDMA pretreatment. Pretreat
with MDMA significantly reduced the increase in locor
activity produced by *S*-MDMA, particularly during the
90-min time interval (time × pretreatment × treatment i
action: $F = 5.93$; dF = 11,407; $P < .0001$) (fig. 4), and te

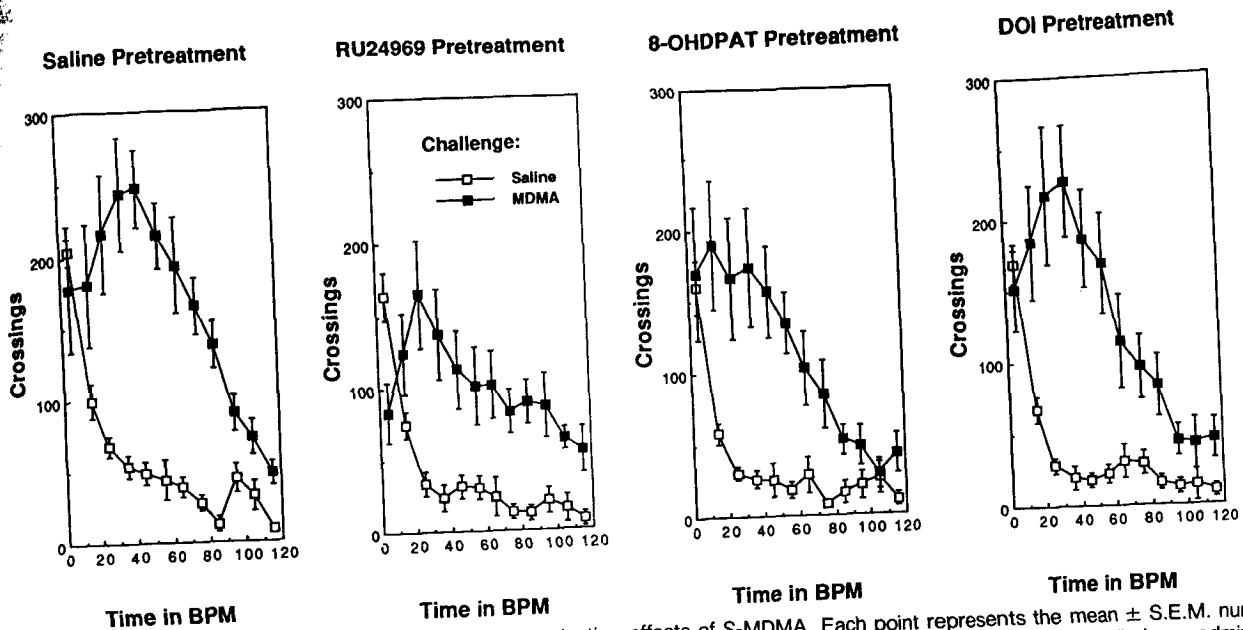

Fig. 1. Effect of repeated 5-HT agonist pretreatment on the activating effects of *S*-MDMA. Each point represents the mean ± S.E.M. number of crossings per 10-min interval for seven to eight animals. Saline, 8-OH-DPAT (1.0 mg/kg), RU24969 (2.5 mg/kg) or DOI (1.0 mg/kg) was administered by s.c. injection every 12 hr for 3 days. Saline (open symbols) or 3.0 mg/kg *S*-MDMA (closed symbols) was administered by s.c. injection 36 hr after the last pretreatment injection. Animals were placed into the BPM 10 min after treatment.

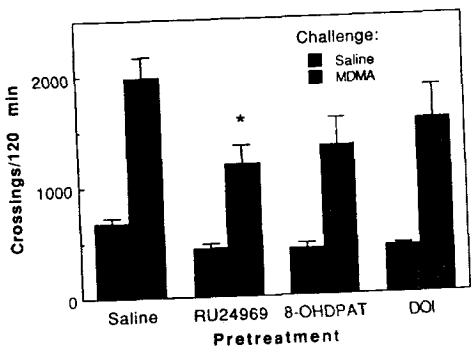

Fig. 2. Effect of repeated 5-HT agonist pretreatment on the activating effects of *S*-MDMA. Each bar represents the mean ± S.E.M. number of crossings per 120-min interval for seven to eight animals. Saline, 8-OH-DPAT (1.0 mg/kg), RU24969 (2.5 mg/kg) or DOI (1.0 mg/kg) was administered by s.c. injection every 12 hr for 3 days. Saline or 3.0 mg/kg *S*-MDMA was administered by s.c. injection 36 hr after the last pretreatment injection. Animals were placed into the BPM 10 min after treatment. *Significant effect of pretreatment, $P < .05$.

($P < .10$) to reverse the *S*-MDMA-induced decrease in the *d* statistic (table 2). A significant effect of MDMA pretreatment on crossings ($F = 6.51$; dF = 1,37; $P < .05$) reflects the fact that MDMA-pretreated control animals were less active than saline-pretreated animals. MDMA pretreatment did not alter the number of hole-pokes exhibited by control animals and did not antagonize the reduction of hole-pokes produced by *S*-MDMA (table 2). MDMA pretreatment did reduce the number of rearings exhibited by control animals ($F = 9.09$; dF = 1,37; $P < .01$) and antagonized both the initial suppression of rearings and the later increase in rearings produced by *S*-MDMA (pretreatment × treatment interaction: $F = 5.96$; dF = 11,407; $P < .0001$) (table 2).

MDMA pretreatment also antagonized the increase in locomotor activity produced by RU24969 ($F = 13.92$; dF = 1,38; $P < .001$) (fig. 4). The present study revealed no effect of MDMA pretreatment on the suppression of hole-pokes produced by RU24969 (table 2). However, MDMA pretreatment slightly reversed the suppression of rearings ($F = 5.80$; dF = 1,38; $P < .05$) and the reduction in the *d* statistic ($F = 5.07$; dF = 1,38; $P < .05$) induced by RU24969 (table 2).

In contrast to the effects on MDMA-induced and RU24969-induced locomotor hyperactivity, MDMA pretreatment significantly increased the locomotor activation produced by *S*-amphetamine ($F = 22.55$; dF = 1,38; $P < .0001$). This dose of *S*-amphetamine produced locomotor hyperactivity in control animals that was similar in magnitude to that produced by the test dose of *S*-MDMA (fig. 4). Furthermore, MDMA pretreatment increased the number of hole-pokes exhibited by *S*-amphetamine-treated animals ($F = 4.72$; dF = 1,38; $P < .05$) (table 2). Although the significant suppression of rearings by MDMA pretreatment was evident after *S*-amphetamine administration, no interaction between MDMA pretreatment and *S*-amphetamine treatment for rearings was evident (table 2).

Persistent effects of MDMA pretreatment. The locomotor-activating effects of *S*-MDMA were still evident when animals were retested 21 days after the first test session (fig. 5). A significant interaction between pretreatment, treatment and time for crossings ($F = 6.56$; dF = 5,150; $P < .0001$) suggested a persistent effect of MDMA pretreatment, and inspection of the data in 30-min intervals suggested that the hyperactivity exhibited by *S*-MDMA-treated animals during the 30- to 60-min time interval was attenuated in the MDMA-pretreated group (fig. 5). However, the *post hoc* comparison between the saline- and MDMA-pretreated groups was not significant. Furthermore, the locomotor hyperactivity induced by RU24969 was unaltered 21 days after MDMA pretreatment (fig. 6). No interactions between MDMA pretreatment and *S*-MDMA or RU24969 treatments were observed for hole-pokes, rearings or spatial *d* (data not shown).

Biochemical effects of MDMA pretreatment. Confirming previous studies (Battaglia *et al.*, 1988), repeated adminis-

TABLE 1

Effects of *S*-MDMA on investigatory behaviors and spatial patterning of exploration 36 hr after repeated administration of RU249 8-OH-DPAT or DOI

	Pretreatment/Treatment (*n*)			
	Saline/Saline (8)	RU24969/Saline (8)	8-OH-DPAT/Saline (7)	*DOI*/Saline (7)
Hole-pokes[a]				
0–30	143.9 ± 13.4	76.6 ± 7.9**	87.0 ± 7.2**	94.7 ± 8.3**
30–60	90.5 ± 20.8	35.0 ± 7.4*	35.0 ± 8.4	34.6 ± 10.2*
60–90	55.3 ± 10.8	19.8 ± 5.7*	35.6 ± 18.3	63.0 ± 16.9
90–120	58.4 ± 10.5	17.0 ± 6.9	41.0 ± 14.0	31.7 ± 11.0
Rearings[a]				
0–30	68.0 ± 14.9	37.0 ± 12.3	48.7 ± 14.5	33.9 ± 15.7
30–60	18.1 ± 6.0	7.6 ± 3.5	8.7 ± 5.9	3.3 ± 2.0
60–90	6.6 ± 2.1	6.0 ± 3.3	9.3 ± 3.8	9.0 ± 4.6
90–120	10.6 ± 4.6	3.6 ± 2.3	7.1 ± 3.7	3.4 ± 1.6
Spatial *d*[a]				
0–60	1.64 ± 0.01	1.69 ± 0.01	1.64 ± 0.01	1.63 ± 0.02
	Pretreatment/Treatment (*n*)			
	Saline/*S*-MDMA (8)	RU24969/*S*-MDMA (8)	8-OH-DPAT/*S*-MDMA (7)	DOI/*S*-MDMA (7)
Hole-pokes[a]				
0–30	52.4 ± 8.1[b]	41.8 ± 6.4	52.3 ± 8.6	46.4 ± 5.6[c]
30–60	80.5 ± 10.4	34.6 ± 5.4	51.4 ± 14.2	59.1 ± 8.1
60–90	83.6 ± 6.1	42.5 ± 6.1	63.1 ± 19.8	108.4 ± 27.9
90–120	91.6 ± 17.7	51.8 ± 7.0	39.0 ± 6.2*	71.3 ± 23.2
Rearings[a]				
0–30	6.1 ± 2.5[b]	3.8 ± 1.9	9.3 ± 2.4	4.4 ± 2.0
30–60	48.5 ± 15.2	9.8 ± 3.9*	18.3 ± 6.5	18.7 ± 6.1
60–90	66.3 ± 16.2[b]	12.6 ± 5.0**	12.6 ± 5.0**	28.6 ± 7.8
90–120	33.4 ± 10.9	14.9 ± 6.8	22.3 ± 9.5	11.9 ± 4.4
Spatial *d*[a]				
0–60	1.53 ± 0.04	1.54 ± 0.04	1.59 ± 0.07	1.56 ± 0.04

[a] Values are mean ± S.E.M.
[b] Significant effect of *S*-MDMA treatment, $P < .01$.
[c] Significant effect of *S*-MDMA treatment, $P < .05$.
* Significant effect of pretreatment, $P < .05$; ** significant effect of pretreatment, $P < .01$.

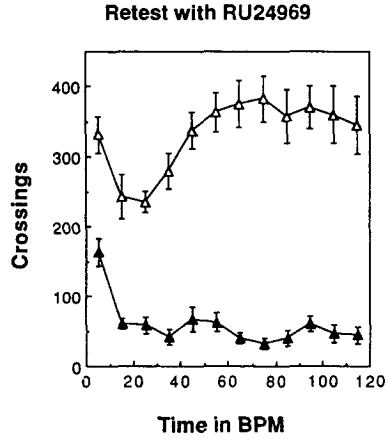

Fig. 3. Tolerance to the locomotor-activating effects of RU24969. Each point represents the mean ± S.E.M. number of crossings per 10-min interval for eight animals. Saline (open symbols) or RU24969 (closed symbols) pretreatments were administered as described in figure 1. After receiving saline and being observed for 2 hr in the BPM, each rat received 2.5 mg/kg RU24969 by s.c. injection and was placed into the BPM 10 min after treatment.

tration of MDMA produced a persistent reduction of 5-HT levels in the hippocampus ($F = 8.91$; dF = 1,18; $P < .01$) and striatum ($F = 24.11$; dF = 1,18; $P < .001$) (table 3). In MDMA-pretreated animals, the primary 5-HT metabolite 5-HIAA was also reduced in both hippocampus ($F = 21.79$; dF = 1,18; $P < .001$) and striatum ($F = 21.38$; dF = 1,18; $P < .001$). No significant effects on levels of DA, DOPAC, HVA or NE were observed in either hippocampus or striatum (table 3).

Discussion

The primary findings of the present study were that develops to the locomotor-activating effects of both t releasing drug *S*-MDMA and the direct 5-HT_{1B} recept RU24969 and that rats exhibit reciprocal cross-tole tween MDMA and RU24969. The specificity of tl tolerance was confirmed by the observation that repe treatment with 8-OH-DPAT, a selective agonist a receptors, or with DOI, a selective agonist at 5-HT receptors, did not reduce the response to *S*-MDMA. C the selectivity of cross-tolerance was indicated by the animals made tolerant to the stimulant-like effe MDMA displayed an augmented hyperactivity respo catecholamine-releasing agent *S*-amphetamine. Thu comotor-activating effects of *S*-MDMA and RU2496 parallel regulation during repeated drug pretreatmen data suggest that *S*-MDMA and RU24969 produce hy ity in rats *via* a common mechanism.

Pretreatment with RU24969, but not with 8-OH-D DOI, antagonized the locomotor hyperactivity produ MDMA. The *S*-MDMA-induced increase in rearin the second hour was attenuated by all three 5-HT pretreatments, suggesting an effect of repeated psy drug administration that was not related to stimula particular receptor subtype. The RU24969-pretreate also exhibited a robust tolerance to RU24969. It is that RU24969-induced locomotor hyperactivity res activation of 5-HT_{1B} receptors that are postsynaptic nergic neurons (Oberlander *et al.*, 1986, 1987; Trick

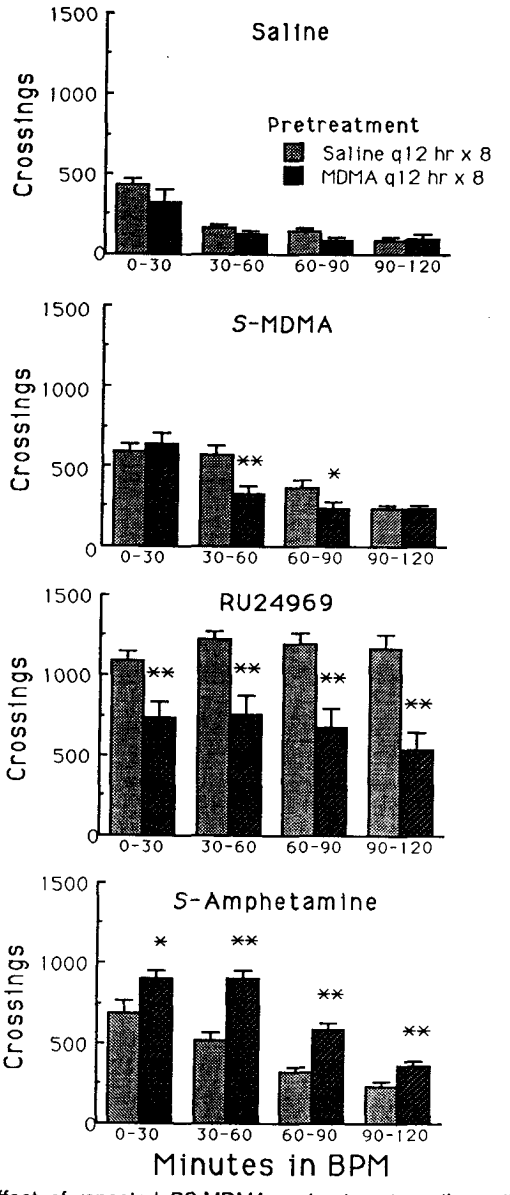

Fig. 4. Effect of repeated *RS*-MDMA pretreatment on the activating effects of *S*-MDMA, RU24969 and amphetamine. Each bar represents the mean ± S.E.M. number of crossings per 30-min interval for 9 to 11 animals. Saline or *RS*-MDMA was administered by s.c. injection every 12 hr for 4 days. Saline, 3.0 mg/kg *S*-MDMA, 2.5 mg/kg RU24969 or 1.0 mg/kg *S*-amphetamine was administered by s.c. injection 36 hr after the last pretreatment injection. Animals were placed into the BPM 10 min after treatment. With the exception of the saline-pretreated, MDMA-challenged animals during the 0- to 30-min interval, all the drug-treated groups differed significantly from controls at all time points. *Significant interaction between pretreatment and treatment, $P < .05$; **significant interaction between pretreatment and treatment, $P < .01$.

al., 1986). Previous work has found that animals made tolerant to RU24969 exhibited unaltered motoric responses to amphetamine or 8-OH-DPAT (Oberlander *et al.*, 1987), consistent with the hypothesis that repeated RU24969 administration results in a selective desensitization of 5-HT_{1B} receptors. Taken together with previous studies indicating that *S*-MDMA-induced hyperactivity is dependent upon release of 5-HT (Callaway *et al.*, 1990), cross-tolerance between *S*-MDMA and RU24969 supports the conclusion that *S*-MDMA produces its major behavioral effects by releasing endogenous 5-HT that in turn stimulates 5-HT_{1B} receptors. Interestingly, RU24969 but not 8-OH-DPAT substitutes for the MDMA congener and selective 5-HT releaser *S*-(+)-N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine in drug discrimination studies (Nichols and Oberlender, 1990).

The regimens of DOI and 8-OH-DPAT used were believed to be sufficient to induce tolerance to activation of 5-HT_2 and 5-HT_{1A} receptors, respectively. For example, DOI shares 5-HT_2 agonist properties with other hallucinogens and has acute behavioral effects in rats that resemble the effects of LSD in the same paradigm used in the present study (Wing *et al.*, 1990). Rapid tolerance to the behavioral effects of hallucinogens can be detected even after a single drug administration (Adams and Geyer, 1985), and this tolerance is associated with down-regulation and subsensitivity of 5-HT_2 receptors (Buckholtz *et al.*, 1985). As few as two injections of the chemically related hallucinogen 2,5-dimethoxy-4-methyl-amphetamine (2.5 mg/kg) can produce down-regulation and behavioral subsensitivity of 5-HT_2 receptors without altering binding parameters of other receptor subtypes (Leysen *et al.*, 1989). Continuous infusions of low doses (0.35 mg/kg/day) of the DOI congener 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane reduces neuroendocrine and ligand-binding assays of 5-HT_2 receptor activation within 1 to 2 days (Owens *et al.*, 1991). The fact that eight daily administrations of DOI (1.0 mg/kg) significantly reduce 5-HT_2 binding sites in rat cortex (McKenna *et al.*, 1989) indicates that DOI shares the pharmacological properties of its congeners. Similarly, long-lasting tolerance to the hyperphagia induced by 8-OH-DPAT develops even after a single injection of 1.0 mg/kg 8-OH-DPAT (Kennett *et al.*, 1987). The forepaw-treading, flat body posture and hypothermia induced by 8-OH-DPAT also exhibit tolerance after one to three injections of 1.0 mg/kg 8-OH-DPAT (Larsson *et al.*, 1990). The significant effects of repeated 8-OH-DPAT administration on the activity of control animals in the present study (figs. 1 and 2, table 1) confirm the ability of this drug regimen to produce lasting effects. These previous studies thus indicate that the present regimens were sufficient to detect any interactions between desensitization of 5-HT_{1A} or 5-HT_2 receptors and the behavioral response to *S*-MDMA.

Pretreatment with MDMA reduced the hyperactivity produced by both *S*-MDMA and RU24969 (fig. 4). In contrast, MDMA pretreatment produced no reduction of the hyperactivity induced by a dose of amphetamine that produced approximately the same level of activity in control animals as did 3.0 mg/kg *S*-MDMA. In fact, MDMA pretreatment augmented the response to *S*-amphetamine, primarily by increasing locomotor activity (fig. 4). This pattern of activity is consistent with a left-shift of the dose-response for *S*-amphetamine in this paradigm (Geyer *et al.*, 1987). Because depletion of 5-HT can increase certain aspects of the behavioral response to amphetamine (Gately *et al.*, 1986), MDMA-induced 5-HT depletion (table 3) may explain the augmented amphetamine response. Alternatively, previous exposure to catecholamine-releasing drugs is known to augment the behavioral effects of amphetamine (Kolta *et al.*, 1985), and the catecholamine-releasing properties of MDMA may be sufficient to evoke this behavioral sensitization. In any event, these results suggest that the ability of MDMA pretreatment to reduce the response to *S*-MDMA or RU24969 was pharmacologically specific and not attributable to impaired motor function or insurmountable fatigue.

TABLE 2

Effects of *S*-MDMA, RU24969 or *S*-amphetamine on investigatory behaviors and spatial patterning of exploration 36 hr after twice-daily administration of 10 mg/kg MDMA for 4 days

	Pretreatment/Treatment (*n*)			
	Saline/Saline (11)	Saline/*S*-MDMA (11)	Saline/RU24969 (11)	Saline/*S*-Amphetamine (11)
Hole-pokes[a]				
0–30	173.2 ± 24.8	59.8 ± 7.1[b]	30.0 ± 3.6[b]	127.5 ± 20.4
30–60	113.3 ± 17.3	135.9 ± 27.8	32.5 ± 8.8[b]	195.9 ± 26.9
60–90	99.7 ± 13.8	138.7 ± 24.2	34.5 ± 9.6[b]	196.3 ± 36.1
90–120	66.0 ± 24.5	163.0 ± 20.5[b]	28.2 ± 9.1	148.3 ± 20.8
Rearings[a]				
0–30	121.1 ± 15.6	5.4 ± 1.8[b]	0.3 ± 0.2[b]	95.3 ± 23.9
30–60	48.2 ± 8.9	54.6 ± 8.4	0.0 ± 0.0[b]	89.6 ± 21.5
60–90	28.1 ± 5.7	78.2 ± 15.3[b]	0.7 ± 0.7[c]	67.0 ± 8.6[b]
90–120	15.1 ± 5.6	80.5 ± 14.5[b]	1.2 ± 0.5	64.5 ± 9.4[b]
Spatial *d*[a]				
0–60	1.63 ± 0.03	1.49 ± 0.10[b]	1.24 ± 0.03[b]	1.62 ± 0.05
	Pretreatment/Treatment (*n*)			
	MDMA/Saline (10)	MDMA/*S*-MDMA (9)	MDMA/RU24969 (10)	MDMA/*S*-Amphetamine (10)
Hole-pokes[a]				
0–30	131.0 ± 12.3	93.2 ± 8.3	45.8 ± 10.7[b]	205.2 ± 37.7
30–60	59.8 ± 9.9	82.7 ± 15.7	31.3 ± 6.4	370.5 ± 123.8*[b]
60–90	75.8 ± 13.4	108.7 ± 31.0	24.7 ± 7.7[c]	295.7 ± 67.8[b]
90–120	52.9 ± 10.2	131.7 ± 14.3[c]	22.8 ± 9.3	246.2 ± 36.0*[b]
Rearings[a]				
0–30	83.2 ± 13.9	41.7 ± 6.8	0.5 ± 0.5[b]	59.3 ± 16.8
30–60	19.5 ± 7.0	31.1 ± 8.7	0.3 ± 0.2	30.7 ± 8.1*
60–90	10.6 ± 5.1	22.0 ± 5.2**	9.7 ± 9.6	35.8 ± 11.3*
90–120	14.7 ± 5.9	35.1 ± 5.6**	6.3 ± 6.3	24.7 ± 6.5**
Spatial *d*[a]				
0–60	1.68 ± 0.04	1.59 ± 0.02	1.39 ± 0.15**[b]	1.61 ± 0.03

[a] Values are mean ± S.E.M.
[b] Significant effect of treatment, $P < .01$.
[c] Significant effect of treatment, $P < .05$.
* Significant effect of MDMA pretreatment, $P < .05$; ** significant effect of MDMA pretreatment, $P < .01$.

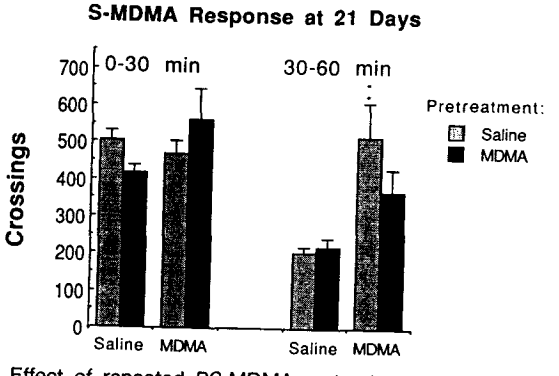

Fig. 5. Effect of repeated *RS*-MDMA pretreatment on the activating effects of *S*-MDMA at 21 days. The mean ± S.E.M. number of crossings per 30-min interval are indicated. Animals were pretreated with saline or *RS*-MDMA and tested with saline or *S*-MDMA. After 21 days, saline or *S*-MDMA (3.0 mg/kg) was administered by s.c. injection 10 min before placing animals into the BPM. **Significant effect of MDMA treatment, $P < .01$.

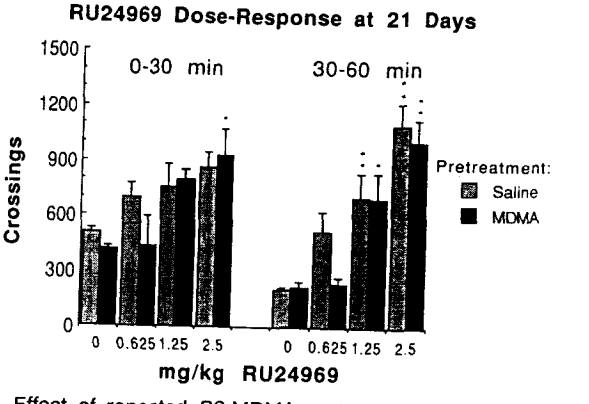

Fig. 6. Effect of repeated *RS*-MDMA pretreatment on the activating effects of RU24969 at 21 days. The mean ± S.E.M. number of crossings per 30-min interval are indicated. Animals were pretreated with saline or *RS*-MDMA and tested with saline or RU24969. After 21 days, saline or RU24969 (0.625, 1.25 or 2.5 mg/kg) was administered by s.c. injection 10 min before placing animals into the BPM. *Significant effect of RU24969 treatment, $P < .05$; **significant effect of RU24969 treatment, $P < .01$.

Because *S*-MDMA-induced hyperactivity is dependent on presynaptic 5-HT (Callaway *et al.*, 1990), depletion of 5-HT after repeated MDMA administration may explain some tolerance to the activating effects of *S*-MDMA. However, the depletion of 5-HT by MDMA persists for weeks (table 3; Battaglia *et al.*, 1988), whereas the trend toward a decreased behavioral response to *S*-MDMA did not reach statistical significance at 21 days (fig. 5). Thus, these data are more consistent with a functional desensitization of the postsynaptic 5-HT receptors that mediate the behavioral effects of *S*-MDMA-induced 5-HT release. At the same time, the reduced response to RU24969 suggests that 5-HT_{1B} receptors that mediate the activating effects of RU24969 are also desensitized during the subchronic MDMA regimen. These findings are consistent with the hypothesis that 5-HT released by MDMA reaches and acts upon 5-HT_{1B} receptors. Taken together with the reduced response to *S*-MDMA after repeated RU24969 administration, these data indicate parallel regulation of the 5-HT receptor

TABLE 3
Brain monoamine levels in rats 36 days after twice-daily administration of 10 mg/kg MDMA for 4 days

Brain Region	Monoamine	Concentrations		% Control
		Control[a]	MDMA[a]	
		pmol/mg tissue		
Hippocampus	5-HT	5.1 ± 0.4	3.4 ± 0.5	−33%[b]
	5-HIAA	3.8 ± 0.2	2.2 ± 0.3	−42%[c]
	NE	6.9 ± 0.4	6.4 ± 0.5	
	DA	0.5 ± 0.1	0.4 ± 0.1	
	DOPAC	0.06 ± 0.02	0.05 ± 0.02	
	HVA	0.26 ± 0.03	0.31 ± 0.06	
Striatum	5-HT	6.8 ± 0.2	4.5 ± 0.4	−34%[c]
	5-HIAA	5.7 ± 0.2	3.8 ± 0.4	−33%[c]
	NE	1.8 ± 0.3	1.7 ± 0.3	
	DA	156.3 ± 50.4	213.1 ± 43.0	
	DOPAC	18.2 ± 1.1	17.9 ± 1.0	
	HVA	16.5 ± 1.2	16.9 ± 0.9	

[a] Values are mean ± S.E.M.
[b] Significantly different from saline pretreatment group, $P < .01$.
[c] Significantly different from saline pretreatment group, $P < .001$.

mediating the activating effects of MDMA and the 5-HT_{1B} receptor believed to mediate the activating effects of RU24969.

The present finding of acute tolerance to the locomotor-activating effects of MDMA contrasts with previous reports that sensitization to the activating effects of MDMA occurs with repeated administration (Spanos and Yamamoto, 1989). The opposite finding in the present study may result from the use of different testing environments and dosing schedules (10 mg/kg every 12 hr *vs.* 2.5–7.5 mg/kg every 48 hr). Furthermore, sensitization to the stimulant-like effects of MDMA on schedule-controlled behavior has been reported after a subchronic MDMA regimen similar to that used in the present study (Li *et al.*, 1989). However, tolerance to the MDMA-induced suppression of milk-drinking by fasted rats occurs after repeated daily administration of MDMA (Zacny *et al.*, 1990). These findings suggest that sensitization develops to certain stimulant-like effects of MDMA and that this sensitization is more evident after intermittent MDMA administration and may be restricted to particular behavioral measures. It may also be important to note that this study used *S*-MDMA to assess the effects of a chronic regimen of racemic MDMA. Depending on the contribution of the *R*-isomer, either more or less tolerance to *S*-MDMA might have been observed if *S*-MDMA had been used for the chronic regimen.

Repeated administration of 5-HT receptor agonists or MDMA tended to decrease the exploratory activity of control animals. The diverse pharmacological profiles of these agents makes it difficult to attribute the reduction of investigatory behaviors to desensitization of particular receptors. In contrast, no reductions of investigatory behaviors were observed in this paradigm after repeated administration of LSD in doses that were sufficient to induce tolerance to LSD (Adams and Geyer, 1985), although such a regimen probably desensitizes 5-HT_2 receptors (Buckholtz *et al.*, 1985). As with the direct 5-HT agonists, repeated administration of MDMA produced a reduction in the exploratory activity of control animals when tested 36 hr after the last injection (fig. 4, table 2). Depletion of endogenous 5-HT may contribute to the reduction of activity induced by repeated MDMA administration (Battaglia *et al.*, 1988), because similar reductions of activity are observed in this paradigm 72 hr after administration of the 5-HT-depleting agent *para*-chlorophenylalanine (Callaway *et al.*, 1990). However, the effect of repeated administration of MDMA on control levels of activity was not evident 21 days later (fig. 5), despite persistent reductions in brain 5-HT levels (table 3). Other studies also have failed to detect lasting behavioral deficits associated with depletion of 5-HT after substituted amphetamine administration (Slikker *et al.*, 1989; Nencini *et al.*, 1988), although changes in rat pup vocalization have been observed after MDMA administration in the context of several other measures that were unaffected (Winslow and Insel, 1990). Inasmuch as serotonergic systems are analogous to other monoaminergic systems, the fact that greater than 90% depletion of tissue catecholamines is required to produce functional deficits (Castaneda *et al.*, 1990) suggests that the modest levels of 5-HT depletion induced by MDMA may be insufficient to functionally compromise serotonergic function. Future studies using more specific or selective assays may reveal whether functional alterations of specific serotonergic systems do occur after submaximal 5-HT depletion.

In summary, repeated administration of the 5-HT_{1B} receptor agonist RU24969 produces cross-tolerance to the locomotor-activating effects of *S*-MDMA. Conversely, repeated administration of MDMA produces cross-tolerance to the locomotor-activating effects of RU24969. The present results along with previously reported data suggest that this cross-tolerance is both specific and selective. Other studies indicate that the locomotor-activating effects of MDMA are mediated by 5-HT release and that the activating effects of RU24969 are mediated *via* direct agonist actions at 5-HT_{1B} receptors. The present finding of cross-tolerance between MDMA and RU24969 thus suggests that the activating effects of MDMA result from indirect agonist actions at 5-HT_{1B} receptors.

Acknowledgments

Special thanks to Marc Hare, Heidi Hartson, Diana Martinez, Virginia Masten and Richard Sharp for expert technical support. Thanks to Dr. Martin Paulus for developing the data analysis software and Dr. Lauren Wing for neurochemical analyses.

References

ADAMS, L. M. AND GEYER, M. A.: A proposed animal model for hallucinogens based on LSD's effects on patterns of exploration in rats. Behav. Neurosci. **99:** 881–900, 1985.

BATTAGLIA, G., YEH, S. Y. AND DESOUZA, E. B.: MDMA-induced neurotoxicity: Parameters of degeneration and recovery of brain serotonin neurons. Pharmacol. Biochem. Behav. **29:** 269–274, 1988.

BRADLEY, P. B., ENGEL, G., FENIUK, W., FOZARD, J. R., HUMPHREY, P. P. A., MIDDLEMISS, D. N., MYLECHARANE, E. J., RICHARDSON, B. P. AND SAXENA, P. R.: Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacology **25:** 563–576, 1986.

BUCKHOLTZ, N. S., FREEDMAN, D. X. AND MIDDAUGH, L. D.: Daily LSD administration selectively decreases serotonin$_2$ receptor binding in rat brain. Eur. J. Pharmacol. **109:** 421–425, 1985.

CALLAWAY, C. W., JOHNSON, M. P., GOLD, L. H., NICHOLS, D. E. AND GEYER, M. A.: Amphetamine derivatives produce locomotor hyperactivity by acting as indirect serotonin agonists. Psychopharmacology **104:** 293–301, 1991.

CALLAWAY, C. W., REMPEL, N., PENG, R. Y. AND GEYER, M. A.: Serotonin 5-HT1-like receptors mediate hyperactivity in rats induced by 3,4-methylenedioxymethamphetamine. Neuropsychopharmacology **7:**113–127, 1992.

CALLAWAY, C. W., WING, L. AND GEYER, M. A.: Serotonin release contributes to the stimulant effects of 3,4,-methylenedioxymethamphetamine in rats. J. Pharmacol. Exp. Ther. **254:** 456–464, 1990.

CASTANEDA, E., WHISHAW, I. Q. AND ROBINSON, T. E.: Changes in striatal dopamine neurotransmission assessed with microdialysis following recovery from a bilateral 6-OHDA lesion: Variation as a function of lesion size. J. Neurosci. **10:** 1847–1854, 1990.

DIXON, W. J.: BMDP Biomedical Computer Programs. University of California Press, Los Angeles, CA, 1990.

EVENDEN, J. L. AND ANGEBY-MOLLER, K.: Effects of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) on locomotor activity and rearing of mice and rats. Psychopharmacology **102:** 485–491, 1990.

GATELY, P. F., SEGAL, D. S. AND GEYER, M. A.: The behavioral effects of depletions of brain serotonin induced by 5,7-dihydroxytryptamine vary with time after administration. Behav. Neural Biol. **45:** 31–42, 1986.

GEYER, M. A., RUSSO, P. AND MASTEN, V.: Multivariate assessment of locomotor behavior: Pharmacological and behavioral analyses. Pharmacol. Biochem. Behav. **28:** 393–399, 1986.

GEYER, M. A., RUSSO, P. V., SEGAL, D. S. AND KUCZENSKI, R.: Effects of apomorphine and amphetamine on patterns of locomotor and investigatory behavior in rats. Pharmacol. Biochem. Behav. **28:** 393–399, 1987.

GOLD, L. H., KOOB, G. F. AND GEYER, M. A.: Stimulant and hallucinogenic behavioral profiles of 3,4-methylenedioxymethamphetamine and N-ethyl-3,4-methylenedioxyamphetamine in rats. J. Pharmacol. Exp. Ther. **247:** 547–555, 1988.

KENNETT, G. A., MARCOU, M., DOURISH, C. T. AND CURZON, G.: Single administration of 5-HT_{1A} agonists decreases 5-HT_{1A} presynaptic, but not postsynaptic receptor-mediated responses: Relationship to antidepressant-like action. Eur. J. Pharmacol. **138:** 53–60, 1987.

KOLTA, M. G., SHREVE, P. AND URETSKY, N. J.: Effect of methylphenidate pretreatment on the behavioral and biochemical responses to amphetamine. Eur. J. Pharmacol. **117:** 279–282, 1985.

LARSSON, L. G., RENYI, L., ROSS, S. B., SVENSSON, B. AND ANGEBY-MOLLER, K.: Different effects on the responses of functional pre- and postsynaptic 5-HT_{1A} receptors by repeated treatment of rats with the 5-HT_{1A} receptor agonist 8-OH-DPAT. Neuropharmacology **29:** 85–91, 1990.

LEYSEN, J. E., JANSSEN, P. F. M. AND NIEMEGEERS, C. J. E.: Rapid desensitization and down-regulation of 5-HT_2 receptors by DOM treatment. Eur. J. Pharmacol. **163:** 145–149, 1989.

LI, A. A., MAREK, G. J., VOSMER, G. AND SEIDEN, L. S.: Long-term central 5-HT depletion resulting from repeated administration of MDMA enhances the effects of single administration of MDMA on schedule-controlled behavior of rats. Pharmacol. Biochem. Behav. **33:** 641–648, 1989.

MCKENNA, D. J., NAZARALI, A. J., HIMENO, A. AND SAAVEDRA, J. M.: Chronic treatment with (±)DOI, a psychotomimetic 5-HT_2 agonist, downregulates 5-HT_2 receptors in rat brain. Neuropsychopharmacology **2:** 81–87, 1989.

MITTMAN, S. M. AND GEYER, M. A.: Effects of 5-HT_{1A} agonists on locomotor and investigatory behaviors in rats differ from those of hallucinogens. Psychopharmacology **98:** 321–329, 1989.

NENCINI, P., WOOLVERTON, W. L. AND SEIDEN, L. S.: Enhancement of morphine-induced analgesia after repeated injections of methylenedioxymethamphetamine. Brain Res. **457:** 136–142, 1988.

NICHOLS, D. E., HOFFMAN, A. J., OBERLENDER, R. A., JACOB, P. III AND SHULGIN, A. T.: Derivatives of 1-(1,3-benzodioxol-5-yl)-2-butanamine: Representatives of a novel therapeutic class. J. Med. Chem. **29:** 2009–2015, 1986.

NICHOLS, D. E. AND OBERLENDER, R.: Structure-activity relationships of MDMA and related compounds: A new class of psychoactive drugs. Ann. N.Y. Acad. Sci. **600:** 613–625, 1990.

OBERLANDER, C., BLAQUIERE, B. AND PUJOL, J. F.: Distinct functions for dopamine and serotonin in locomotor behaviour: Evidence using the 5-HT_1 agonist RU 24969 in globus pallidus-lesioned rats. Neurosci. Lett. **67:** 113–118, 1986.

OBERLANDER, C., DEMASSEY, Y., VERDU, A., VAN DE VELDE, D. AND BARDELAY, C.: Tolerance to the 5-HT_1 agonist RU24969 and effects on dopaminer behavior. Eur. J. Pharmacol. **139:** 205–214, 1987.

OWENS, M. J., KNIGHT, D. L., RITCHIE, J. C. AND NEMEROFF, C. B.: The hydroxytryptamine$_2$ agonist, (±)-1-(2,5-dimethoxy-4-bromophenyl)-2-amin propane stimulates the hypothalamic-pituitary-adrenal (HPA) axis. II. Bi chemical and physiological evidence for the development of tolerance af chronic administration. J. Pharmacol. Exp. Ther. **256:** 795–800, 1991.

PAULUS, M. AND GEYER, M. A.: A temporal and spatial scaling hypothesis f the behavioral effects of psychostimulants. Psychopharmacology **104:** 6–1 1991.

PAZOS, A., HOYER, D. AND PALACIOS, J. M.: The binding of serotonergic ligand to the porcine choroid plexus: Characterization of a new type of serotoni recognition site. Eur. J. Pharmacol. **106:** 539–546, 1984.

PEDIGO, N. W., YAMAMURA, H. J. AND NELSON, D. L.: Discrimination of multipl ^{3}H-5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain J. Neurochem. **36:** 220–226, 1981.

PEROUTKA, S. J. AND SNYDER, S. H.: Regulation of serotonin$_2$ (5-HT_2) receptors labeled with [^{3}H]spiroperidol by chronic treatment with the antidepressant amitryptyline. J. Pharmacol. Exp. Ther. **215:** 582–587, 1980.

REMPEL, N. L., CALLAWAY, C. W. AND GEYER, M. A.: Serotonin$_{1B}$ receptor activation mimics behavioral effects of presynaptic serotonin release. Neuropsychopharmacology, in press, 1992.

SAVAGE, D. D., MENDELS, J. AND FRAZER, A.: Monoamine oxidase inhibitors and serotonin uptake inhibitors: Differential effects on [^{3}H]serotonin binding sites in rat brain. J. Pharmacol. Exp. Ther. **212:** 259–263, 1980.

SLIKKER, W., HOLSON, R. R., ALI, S. F., KOLTA, M. G., PAULE, M. G., SCALLET, A. C., MCMILLAN, D. E., BAILEY, J. R., HONG, J. S. AND SCALZO, F. M.: Behavioral and neurochemical effects of orally administered MDMA in the rodent and nonhuman primate. Neurotoxicology **10:** 529–542, 1989.

SPANOS, L. J. AND YAMAMOTO, B. K.: Acute and subchronic effects of methylenedioxymethamphetamine (±MDMA) on locomotion and serotonin syndrome behavior in the rat. Pharmacol. Biochem. Behav. **32:** 835–840, 1989.

TRICKLEBANK, M. D., MIDDLEMISS, D. N. AND NEILL, J.: Pharmacological analysis of the behavioural and thermoregulatory effects of the putative 5-HT receptor agonist, RU24969, in the rat. Neuropharmacology **25:** 877–886, 1986.

WING, L. L., TAPSON, G. S. AND GEYER, M. A.: 5-HT_2 mediation of acute behavioral effects of hallucinogens in rats. Psychopharmacology **100:** 417–425, 1990.

WINSLOW, J. T. AND INSEL, T. R.: Serotonergic modulation of rat pup ultrasonic vocal development: Studies with 3,4-methylenedioxymethamphetamine. J. Pharmacol. Exp. Ther. **254:** 212–220, 1990.

ZACNY, J. P., VIRUS, R. M. AND WOOLVERTON, W. L.: Tolerance and cross-tolerance to 3,4-methylenedioxymethamphetamine (MDMA), methamphetamine and methylenedioxyamphetamine. Pharmacol. Biochem. Behav. **35:** 637–642, 1990.

Send reprint requests to: Mark A. Geyer, Ph.D., Department of Psychiatry, 0804, University of California, San Diego, La Jolla, CA 92093-0804.