

Pharmacological Studies of the Acute and Chronic Effects of (+)-3,4-Methylenedioxymethamphetamine on Locomotor Activity: Role of 5-Hydroxytryptamine_{1A} and 5-Hydroxytryptamine_{1B/1D} Receptors¹

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ABSTRACT

The 5-hydroxytryptamine_{1B/1D} (5-HT_{1B/1D}) antagonist 2'-methyl-4'-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-amide (GR 127935) and 5-HT_{1A} antagonist *N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY 100635) were used to assess whether hyperactivity induced by 3 mg/kg (+)-3,4-methylenedioxymethamphetamine [(+)-MDMA] is mediated by 5-HT_{1B/1D} and/or 5-HT_{1A} receptors. Activity in the periphery and center of an open field as well as rearing activity were measured in photobeam monitors. (+)-MDMA-induced peripheral and central activities were blocked by GR 127935 (0.3, 0.625, 1.25, and 2.5 mg/kg); central hyperactivity was blocked by 0.1, 0.3, and 0.625 mg/kg GR 127935. WAY 100635 (0.5–2 mg/kg) had little effect on (+)-MDMA-induced activity except for an enhancement of central activity at one dose (0.5 mg/kg). Central activity induced by (+)-MDMA increased from day 1 to day 5 of treatment with (+)-MDMA (3 mg/kg), whereas peripheral, central,

and rearing activity significantly increased in (+)-MDMA-treated rats pretreated daily with GR 127935 (2.5 mg/kg). Withdrawal from (+)-MDMA, but not GR 127935 + (+)-MDMA, pretreatment was associated with heightened hyperactivity induced by the 5-HT_{1B/1A} agonist RU 24969 (2 mg/kg i.p.); treatments were not associated with alterations in 5-HT and 5-hydroxyindoleacetic acid content or turnover in frontal cortex. These data support a role for 5-HT_{1B/1D} in mediating the acute hyperactivity evoked by (+)-MDMA. The development of sensitization to (+)-MDMA was associated with supersensitivity to a 5-HT_{1B/1A} agonist, suggesting that these receptors may contribute to sensitization. However, sensitization to (+)-MDMA developed even under conditions of 5-HT_{1B/1D} receptor blockade, which is somewhat counter to this speculation. Perhaps, under circumstances of continued 5-HT_{1B/1D} blockade, other mechanisms (e.g., dopamine) predominate in the progressive enhancement of behavior with repeated (+)-MDMA treatment.

3,4-Methylenedioxymethamphetamine (MDMA; "ecstasy") elicits a unique constellation of subjective effects that differs from the psychostimulant amphetamine (Peroutka et al., 1988). The behavioral profile for MDMA in animals is also unique in comparison with amphetamine. The hyperactivity induced by MDMA is characterized by increased forward locomotion in the periphery of the chamber, whereas amphetamine evokes hypermotility distributed throughout the entire activity monitor (Gold et al., 1989; Callaway et al., 1990). Decreased investigatory behaviors, e.g., holepokes and rearing activity (Gold et al., 1989; Callaway et al., 1990), and

elicitation of the serotonin (5-HT; 5-hydroxytryptamine) syndrome (Spanos and Yamamoto, 1989) are also observed after MDMA administration. The ability of MDMA to release 5-HT because of a 5-HT-MDMA exchange (Rudnick and Wall, 1992) has been proposed to mediate the MDMA-induced 5-HT syndrome (Spanos and Yamamoto, 1989). However, unlike the case for *d*-amphetamine (Callaway et al., 1990), both the 5-HT and dopamine (DA) systems may mediate hyperactivity induced by MDMA. First, MDMA evokes the release of DA as well as 5-HT in vivo (Yamamoto and Spanos, 1988; White et al., 1994). Second, the pattern of behavior evoked by MDMA is more similar to that induced by substituted amphetamines that enhance 5-HT, but not DA, release than to that evoked by amphetamine, which is predominantly a DA releaser (Callaway et al., 1991). Pretreatment with selective 5-HT reuptake inhibitors, which presumably

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ABBREVIATIONS: (+)-MDMA, (+)-3,4-methylenedioxymethamphetamine; 5-HT, 5-hydroxytryptamine; DA, dopamine; GR 127935, 2'-methyl-4'-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-amide; WAY 100635, *N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridinyl)cyclohexanecarboxamide; 5-HIAA, 5-hydroxyindole acetic acid; VTA, ventral tegmental area; NAC, nucleus accumbens.

block access of MDMA to the 5-HT transporter, antagonized MDMA-induced, but not amphetamine-induced, hyperactivity (Callaway et al., 1990; Callaway et al., 1991). Furthermore, depletion of 5-HT (Callaway et al., 1990) or DA (Gold et al., 1989; but see Callaway et al., 1990) attenuated MDMA-evoked hyperactivity as did antagonists with affinity at either 5-HT or DA receptors (Callaway et al., 1992; Kehne et al., 1996). Thus, MDMA appears to evoke hyperactivity via indirect actions in both the DA and 5-HT systems.

Because of the potentially unique contribution of 5-HT to hyperactivity evoked by MDMA, attempts to identify the 5-HT receptors involved have been made. Subsequent to the release of 5-HT, an indirect activation of 5-HT_{1B} receptors has been proposed as important to its behavioral effects (Callaway et al., 1992; Rempel et al., 1993). This conclusion is based on the observation that 5-HT agonists with affinity for 5-HT_{1B} receptors elicit a behavioral profile similar to MDMA and that ligands that function as nonselective 5-HT_{1B} receptor antagonists block the behavioral activation evoked by MDMA. However, many of the antagonists that have been shown to attenuate MDMA-induced activity (e.g., methiothepin and propranolol) also have appreciable affinity for the 5-HT_{1A} receptor (Callaway et al., 1992; Kehne et al., 1996). Furthermore, the 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin can induce a prominent forward locomotion that is blocked by 5-HT_{1A} antagonists (Kalkman and Soar, 1990). Thus, both 5-HT_{1A} and 5-HT_{1B} receptors may contribute to the locomotor stimulant effects of MDMA, although this hypothesis has not yet been tested with selective antagonists.

Psychostimulants enhance hyperactivity and stereotypy during repeated, intermittent administration ("behavioral sensitization") (Robinson and Berridge, 1993). Demonstrations of sensitization to MDMA exposure are limited. Rats repeatedly treated with MDMA displayed sensitized hyperactivity (Spanos and Yamamoto, 1989; Dafters, 1995; Kalivas et al., 1998), 5-HT syndrome behaviors (Spanos and Yamamoto, 1989), and hyperthermia (Dafters, 1995). However, the development of sensitization to MDMA is not consistently reported; in fact, tolerance and no effect of repeated treatment with MDMA have been observed (Callaway and Geyer, 1992; McNamara et al., 1995).

The present study was designed to analyze the relative importance of 5-HT_{1B/1D} and 5-HT_{1A} receptors in hyperactivity evoked by a low dose of (+)-MDMA; the (+)-MDMA isomer was chosen because of its greater potency in eliciting hyperactivity compared with the (–)-isomer (Paulus and Geyer, 1992). For this investigation, the novel 5-HT_{1B/1D} antagonist 2'-methyl-4'-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-amide (GR 127935; Skingle et al., 1996) and the selective 5-HT_{1A} antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinylethyl]-*N*-2-pyridinyl-cyclohexanecarboxamide (WAY 100635; Forster et al., 1995) were used. The ability of (+)-MDMA (3 mg/kg/day for 5 days) to evoke behavioral sensitization in the presence or absence of GR 127935 was analyzed as was the sensitivity to hyperactivity evoked by 5-HT_{1B/1A} agonist RU 24969 (Callaway et al., 1992) during withdrawal. The dose regimen of MDMA was chosen as likely to induce behavioral sensitization (Spanos and Yamamoto, 1989) but to be subthreshold for elicitation of the 5-HT syndrome (Spanos and Yamamoto, 1989) and induction of 5-HT neurotoxicity (Kalivas et al., 1998). To assure that

this was the case, frontal cortex was collected 24 h after the challenge injection of RU 24969 and assayed for 5-HT and 5-hydroxyindole acetic acid (5-HIAA) content.

Materials and Methods

Animals

Male Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) weighing 225–250g at the beginning of experimental procedures were housed in groups of four in a temperature (21–23°C) and humidity (40–50%) controlled environment for at least 1 week before experiments. Food and water were available ad libitum, except during experimental sessions. Lighting was maintained under a 12-h light/dark cycle (lights on 0700–1900 h). All experimental procedures were performed between 0800 and 1530 h.

Apparatus

Locomotor activity was quantified using an open-field activity system (San Diego Instruments, San Diego, CA) housed within sound-attenuated chambers. Each enclosure consisted of a clear Plexiglas open field (40 × 40 × 40 cm) and a 4 × 4 photobeam matrix located 4 cm above the cage floor. Another horizontal row of 16 photobeams located 16 cm from the floor surface provided each chamber with a measure of rearing activity. Activity recorded in the inner 16 × 16 cm of the open field was counted as central activity, whereas the field bounded by the outer 16-cm band registered peripheral activity. Counts of peripheral and central horizontal activity and rearing activity were made by the control software (Photobeam Activity Software; San Diego Instruments) and stored for subsequent statistical evaluation. Video cameras located above the chambers were used to monitor activity continuously without disruption of behavior.

Behavioral Procedures

Rats were habituated to the test chambers for 3 h/day on the 2 days before the start of the experiment.

Experiment 1. Effects of GR 127935 on Spontaneous and MDMA-Evoked Activity. Each of five groups of rats ($n = 8/\text{group}$) was assigned a particular dose of GR 127935 (0.1, 0.3, 0.625, 1.25, or 2.5 mg/kg s.c.) and tested under each of four conditions consisting of the dose of GR 127935 or β -cyclodextrin vehicle [10% (w/v); 2 ml/kg s.c.] followed by an injection of either (+)-MDMA (3 mg/kg s.c.) or saline [0.9% (w/v); 1 ml/kg s.c.]. Procedurally, rats were placed in the activity monitors for 15 min before an injection of the assigned dose of GR 127935 or vehicle, followed 45 min later by (+)-MDMA or saline. Recording of activity began immediately in 5-min time epochs for 90 min. The presentation of drugs was counterbalanced so that the order of drug exposure was randomized for each individual animal. Tests were spaced such that animals received injections 48 h apart over the course of a 2-week period; the two doses of (+)-MDMA administered were spaced at least 4 days apart.

Experiment 2. Effects of WAY 100635 on Spontaneous and MDMA-Evoked Activity. Each of three groups of rats ($n = 8/\text{group}$) was assigned a particular dose of WAY 100635 (0.5, 1, or 2 mg/kg s.c.) and tested under each of four conditions consisting of the dose of WAY 100635 or saline followed by an injection of either (+)-MDMA or saline. Procedurally, rats were placed in the activity monitors for 30 min before an injection of the assigned dose of WAY 100635 or saline (1 ml/kg s.c.), followed 30 min later by (+)-MDMA (3 mg/kg s.c.) or saline (1 ml/kg s.c.). Recording of activity was conducted as in experiment 1.

Experiment 3. Effects of GR 127935 on Spontaneous and (+)-MDMA-Induced Activity and Behavioral Sensitization. After a 15-min habituation to the activity monitor on each test day, rats were pretreated with GR 127935 (2.5 mg/kg s.c.) or β -cyclodextrin vehicle (2 ml/kg s.c.) 45 min before an injection of (+)-MDMA (3 mg/kg s.c.) or saline (1 ml/kg s.c.). Recording of activity began im-

mediately in 5-min time epochs for 120 min. Animals were exposed to this cotreatment regimen once daily for 5 days, and activity was monitored daily in the test chambers. The four independent groups of rats ($n = 8/\text{group}$) received vehicle + saline, GR 127935 + saline, vehicle + (+)-MDMA, or GR 127935 + (+)-MDMA. Activity was recorded immediately after the administration of (+)-MDMA or vehicle in 5-min time epochs for 120 min.

Twenty-four hours after the last cotreatment injections, rats were rehabilitated to the activity monitors for 60 min before an injection of saline (1 ml/kg s.c.). Recording of activity began immediately in 5-min time epochs for 120 min. This test was included to assess the conditioned activity in response to the test environment. After 48 h of withdrawal, rats were rehabilitated to the test environment for 60 min and challenged subsequently with an injection of RU 24969 (2 mg/kg i.p.). This test was included to assess the sensitivity of 5-HT_{1B/1A} receptors after the chronic cotreatment regimens. Activity was recorded in 5-min time epochs for 105 min beginning 15 min after the RU 24969 injection.

Biochemical Assays

Tissue Preparation. Twenty-four hours after the RU 24969 challenge, rats were anesthetized with chloral hydrate (800 mg/kg i.p.) and decapitated. The frontal cortex was dissected (Heffner et al., 1980), and the tissue was immediately submerged in liquid nitrogen and transferred to storage at -80°C until assay.

Analysis of 5-HT and 5-HIAA Content. Analysis of 5-HT and 5-HIAA content was conducted in the laboratory of Bryan Yamamoto by Wenjun Zhu (Case Western Reserve University, Cleveland, OH). Frontal cortex tissue was homogenized by sonication in 1 ml of cold perchloric acid (0.1 M). Homogenates were centrifuged at 14,500g for 10 min at 12°C , and the resultant supernatants were filtered (0.2 μm syringe filter) and injected into an HPLC with electrochemical detection for 5-HT and 5-HIAA (E.G. and G. Princeton Applied Research electrochemical detector model 400). The analytes were separated with a C18 reversed phase column [100 $\times$ 2 mm i.d., ODS(3), 5 μm particle size; Prodigy, Phenomenex, Torrance, CA] and eluted using a mobile phase consisting of sodium acetate (20 mM), citrate (12.5 mM), methanol (3%, v/v), octane sulfonic acid (26 mg/l) and EDTA (100 mg/l). The column temperature was maintained at 32°C . 5-HT and 5-HIAA were oxidized at a glassy carbon electrode maintained at a potential of 650 mV compared with a Ag/AgCl reference electrode. Protein determinations were made according to the methods of Bradford (1976).

Drugs

β -cyclodextrin (2-hydroxypropyl- β -cyclodextrin) was obtained from Research Biochemicals, Inc. (Natick, MA), GR 127935 was obtained from Glaxo-Wellcome (Ware, Hertfordshire, U.K.), (+)-MDMA was obtained from the National Institutes on Drug Abuse (Research Triangle Park, NC), and WAY 100635 was obtained from Wyeth-Ayerst (Princeton, NJ). Doses refer to the weight of the salt; (+)-MDMA and WAY 100635 were dissolved in 0.9% saline, and GR 127935 was dissolved in 10% β -cyclodextrin in 0.9% saline with mild heating. Verification that the compounds had dissolved was made visually.

Statistical Analyses

Experiments 1 and 2. Total activity counts were summed for each individual rat across the 90-min observation period; data are presented as mean total activity counts ($\pm$ S.E.M.). A one-way repeated measures ANOVA was used to determine the main effect of pretreatment (e.g., vehicle + saline, GR 127935 + saline, vehicle + (+)-MDMA, or GR 127935 + (+)-MDMA) on the dependent measures of total peripheral, central, and vertical activity observed during the 90-min test session. Planned group comparisons were assessed with a least significant difference test with the error rate (α) set at 0.05.

Experiment 3. Total activity counts were summed for each individual rat across the 90-min observation period; data are presented as mean total activity counts ($\pm$ S.E.M.). A one-way ANOVA was used to assess differences in total activity counts for the 90-min test session among the four cotreatment groups on days 1 and 5 and after challenges during withdrawal. A subsequent planned comparisons least significant difference test was performed among treatment groups. A paired Student's *t* test was used to analyze activity counts between days 1 and 5 within each pretreatment group. All statistical analyses were conducted with an error rate (α) set at 0.05.

The content of 5-HT and 5-HIAA in the frontal cortex is presented as nanograms per milligram of protein and was analyzed with a one-way ANOVA for independent measures.

Results

Experiment 1. Effects of GR 127935 on Spontaneous and MDMA-Evoked Activity

The ability of GR 127935 to affect (+)-MDMA-evoked hyperactivity was assessed in six groups of animals, using a repeated measures design. A main effect of pretreatment was evident for peripheral activity in the groups of rats tested with all doses of GR 127935. A main effect of pretreatment was also observed for central activity in the groups of rats treated with 0.1, 0.3, and 0.625 mg/kg GR 127935 (see Table 1 for results of ANOVAs). A significant main effect of pretreatment was evident for rearing activity only in the groups of rats treated with 0.625 mg/kg GR 127935, whereas a trend toward an effect of treatment on rearing activity was observed in the group exposed to 2.5 mg/kg GR 127935 (see Table 1 for results of ANOVAs). Of the five groups of rats ($n = 8$ rats/group) tested with (+)-MDMA after an injection of vehicle, a priori analyses revealed that (+)-MDMA (3 mg/kg; Figs. 1 and 2, left-hatched column) significantly increased peripheral (Figs. 1 and 2, top panels) and central activity (Figs. 1 and 2, middle panels) over basal levels ($p < .05$); rearing activity increased inconsistently across groups (Figs. 1 and 2, bottom panels). Pretreatment with GR 127935 alone (all doses; Figs. 1 and 2, cross-hatched columns) had no significant effect on peripheral (Figs. 1 and 2, top panels), central (Figs. 1 and 2, middle panels), or rearing activity (Figs. 1 and 2, bottom panels). On the other hand, pretreat-

TABLE 1
Spontaneous and MDMA-evoked activity: effects of GR 127935

GR 127935 mg/kg	Peripheral	Central	Rearing
0.1	$F_{3,28} = 4.65, p = .0092$	$F_{3,28} = 6.67, p = .0015$	$F_{3,28} = 1.91, p = .15$
0.3	$F_{3,28} = 12.99, p = .0001$	$F_{3,28} = 6.13, p = .0024$	$F_{3,28} = 1.05, p = .385$
0.625	$F_{3,28} = 11.67, p = .0001$	$F_{3,28} = 7.29, p = .0009$	$F_{3,28} = 5.28, p = .0052$
1.25	$F_{3,28} = 15.70, p = .0001$	$F_{3,28} = 2.06, p = .1281$	$F_{3,28} = 1.15, p = .34$
2.5	$F_{3,28} = 13.07, p = .0001$	$F_{3,28} = 1.40, p = .2633$	$F_{3,28} = 2.64, p = .068$

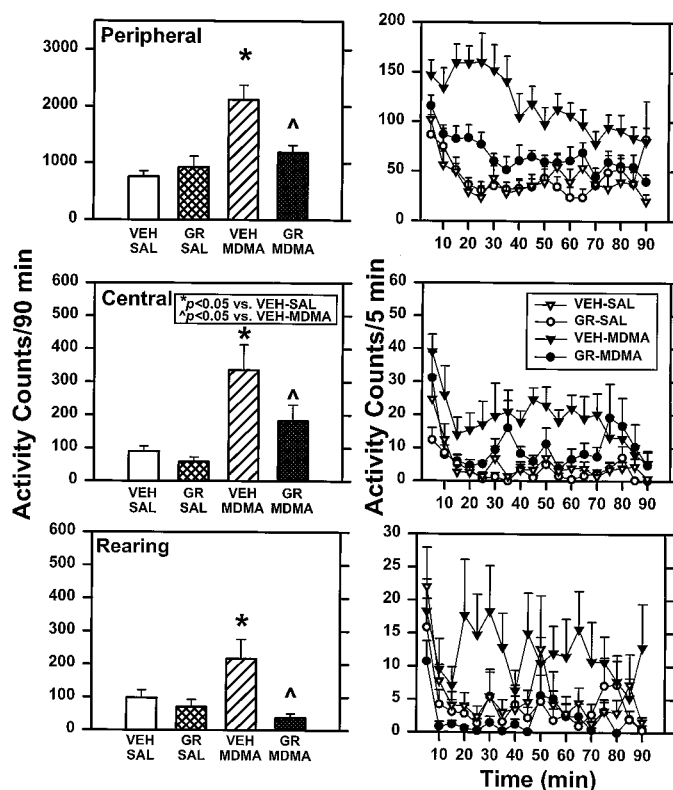


Fig. 1. Effects of acute administration of GR 127935 (0.625 mg/kg) on spontaneous and (+)-MDMA-induced locomotor activity. Fifteen minutes after placement in the activity monitors, rats were injected with GR 127935 (GR; 0.625 mg/kg s.c.) or β -cyclodextrin vehicle (VEH; 10% w/v; 2 ml/kg s.c.) followed 45 min later by (+)-MDMA (MDMA; 3 mg/kg s.c.) or saline (SAL; 1 ml/kg s.c.). Recording of activity began immediately in 5-min time epochs for 90 min. Left, the mean activity counts ($\pm$ S.E.M.) summed over the 90-min period beginning immediately after the second injection (SAL or MDMA; $n = 8$ /group) are shown for the four pretreatment groups: VEH + SAL (open columns), GR + SAL (cross-hatched columns), VEH + MDMA (left-hatched columns), and GR + MDMA (dotted columns). Peripheral, central, and rearing activity are shown in the top, middle, and bottom panels, respectively. *, activity levels that differed significantly from those in VEH + SAL rats. Δ , activity levels in GR + MDMA rats that differed from those in VEH + MDMA rats ($p < .05$). Right, data are presented as mean activity counts ($\pm$ S.E.M.) in each 5-min bin over the 90-min period beginning immediately after the second injection (SAL or MDMA) for the VEH + SAL (∇), GR + SAL ($\circ$), VEH + MDMA ($\blacktriangledown$), and GR + MDMA ($\bullet$) rats.

ment with GR 127935 (Figs. 1 and 2, dotted columns) significantly attenuated peripheral (0.3–2.5 mg/kg; Figs. 1 and 2, top panels) and central activity (0.3 and 0.625 mg/kg; Figs. 1 and 2, middle panels) evoked by (+)-MDMA ($p < .05$). In the one group in which (+)-MDMA increased rearing activity (Fig. 1), GR 127935 (0.625 mg/kg) effectively antagonized this increase ($p < .05$). An example of the timecourse for activity is shown in Fig. 1, right panels.

Experiment 2. Effects of WAY 100635 on Spontaneous and MDMA-evoked Activity

A main effect of pretreatment was evident for peripheral and central activity of all groups pretreated with WAY 100635 (see Table 2 for results of ANOVAs). Of the three groups of rats ($n = 8$ rats/group) tested with (+)-MDMA after an injection of vehicle, (+)-MDMA (3 mg/kg; Fig. 3, left-hatched column) significantly increased peripheral (Fig. 3, top panel) and central activity (Fig. 3, middle panel) over basal levels ($p < .05$); no significant changes in rearing ac-

tivity were observed (Fig. 3, bottom panel). Pretreatment with WAY 100635 alone (Fig. 3, cross-hatched column) did not affect peripheral (Fig. 3, top panel), central (Fig. 3, middle panel), or rearing activity (Fig. 3, bottom panel). (+)-MDMA-induced central activity (Fig. 3, dotted column) increased after pretreatment with WAY 100635 (0.5 mg/kg; $p < .05$; Fig. 3).

Experiment 3. Effects of GR 127935 on Spontaneous and (+)-MDMA-Induced Activity and Behavioral Sensitization

Effects of Pretreatment: Day 1. A major effect of pretreatment on peripheral ($F_{3,28} = 6.51$, $p = .0018$) and central ($F_{3,28} = 4.33$, $p = .0126$), but not rearing ($F_{3,28} = 1.68$, $p = .19$), activity was observed on day 1. Significant increases in peripheral activity (Fig. 4, top panel), but not central (Fig. 4, middle panel), or rearing activity (Fig. 4, bottom panel), were observed after treatment with vehicle + (+)-MDMA compared with vehicle + saline. Although rats treated with vehicle + GR 127935 showed similar activity levels as rats treated with vehicle + saline, pretreatment with GR 127935 significantly attenuated peripheral (Fig. 4, top panel) and central (Fig. 4, middle panel) activity evoked by (+)-MDMA on day 1 ($p < .05$).

Effects of Pretreatment: Day 5. A major effect of pretreatment on peripheral ($F_{3,28} = 13.65$, $p = .0001$) and central ($F_{3,28} = 9.70$, $p = .0001$) activity was observed; a trend toward a major effect of pretreatment on rearing activity was also observed ($F_{3,28} = 2.92$, $p = .051$). Significant increases were observed in peripheral (Fig. 4, top panel) and central (Fig. 4, middle panel) activity after treatment with vehicle + (+)-MDMA compared with vehicle + saline ($p < .05$). As on day 1, rats treated with vehicle + GR 127935 showed similar activity levels as rats treated with vehicle + saline. On day 5, pretreatment with GR 127935 did not significantly attenuate the (+)-MDMA-evoked increase in peripheral (Fig. 4, top panel) or central (Fig. 4, middle panel) activity.

Effects of Pretreatment: Day 1 to Day 5. To determine whether significant changes occurred during the course of the chronic regimen from day 1 to day 5, comparisons of activity levels were made within treatment groups. In rats treated with vehicle + saline or GR 127935 + saline, peripheral, central, and vertical activity did not differ between days 1 and 5. On the other hand, rats treated with vehicle + (+)-MDMA showed enhanced central activity ($p < .05$) and a trend toward enhanced rearing activity ($p = .063$). In rats treated with GR 127935 + (+)-MDMA, peripheral and central ($p < .05$), but not rearing, activity increased between days 1 and 5 ($p < .05$).

Challenge Tests with Saline or RU 24969 during Withdrawal. No major effect of pretreatment was observed for peripheral ($F_{3,26} = 0.60$, $p = .62$), central ($F_{3,26} = 1.87$, $p = .16$), or rearing ($F_{3,26} = 2.22$, $p = .11$) activity upon challenge with saline 24 h after the last pretreatment injection (data not shown). A major effect of pretreatment was observed for peripheral ($F_{3,25} = 3.36$, $p = .0347$), but not central ($F_{3,25} = 1.66$, $p = .20$) or rearing ($F_{3,25} = 0.74$, $p = .53$), activity upon challenge with RU 24969 (2 mg/kg) 48 h after the last pretreatment injection. Enhanced peripheral activity was seen in vehicle + (+)-MDMA, but not GR 127935 + (+)-MDMA, rats compared with vehicle + saline rats ($p < .05$) (Fig. 5).

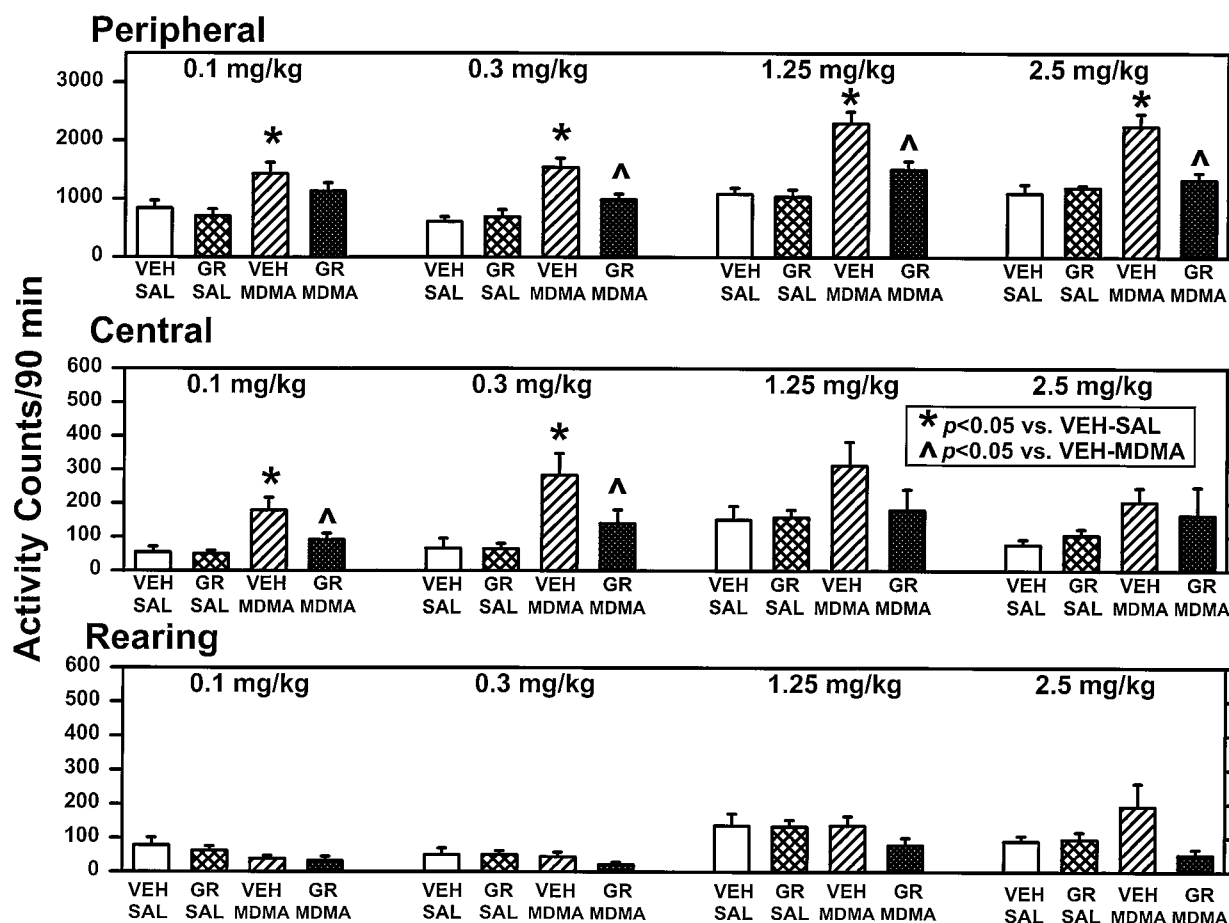


Fig. 2. Effects of acute administration of GR 127935 on spontaneous and (+)-MDMA-induced locomotor activity. Fifteen minutes after placement in the activity monitors, rats were injected with GR 127935 (GR; 0.1, 0.3, 1.25, or 2.5 mg/kg s.c.) or β -cyclodextrin vehicle followed 45 min later by (+)-MDMA (MDMA; 3 mg/kg s.c.) or saline (SAL; 1 ml/kg s.c.). Recording of activity began immediately in 5-min time epochs for 90 min. The mean activity counts ($\pm$ S.E.M.) summed over the 90-min period beginning immediately after the second injection (SAL or MDMA; $n = 8$ /group) are shown for the four pretreatment groups: VEH + SAL (open columns), GR + SAL (cross-hatched columns), VEH + MDMA (left-hatched columns), and GR + MDMA (filled columns) for peripheral (top), central (center) and rearing (bottom) activity. *, activity levels that differed significantly from those in VEH + SAL rats. Δ , activity levels in GR + MDMA rats that differed from those in VEH + MDMA rats ($p < .05$).

TABLE 2

Spontaneous and MDMA-evoked activity: effects of WAY 100635

WAY 100635 mg/kg	Peripheral	Central	Rearing
0.5	$F_{3,28} = 6.77, p = .0014$	$F_{3,28} = 10.98, p = .0001$	$F_{3,28} = 1.52, p = .23$
1.0	$F_{3,28} = 12.51, p = .0001$	$F_{3,28} = 10.40, p = .0001$	$F_{3,28} = 1.88, p = .15$
2.0	$F_{3,28} = 7.29, p = .0009$	$F_{3,28} = 7.29, p = .0009$	$F_{3,28} = 1.94, p = .15$

5-HT and 5-HIAA Determinations in Frontal Cortex.

No main effect of pretreatment on 5-HT content ($F_{3,28} = 0.87, p = .46$), 5-HIAA content ($F_{3,28} = 0.72, p = .54$), or the ratio of 5-HT to 5-HIAA in the frontal cortex ($F_{3,28} = 0.88, p = .4641$) was observed (Table 3).

Discussion

Hyperactivity Induced by Acute (+)-MDMA: Role of 5-HT_{1A} and 5-HT_{1B/1D} Receptors. Consistent with earlier reports, administration of a low dose (3 mg/kg) of (+)-MDMA predominantly increased activity in the periphery of the activity monitor, although increased central activity was also observed. In contrast, (+)-MDMA failed to consistently alter rearing, although depressed rearing activity after this dose of

(+)-MDMA has been reported (see *Introduction*). The 5-HT_{1B/1D} antagonist GR 127935 efficaciously blocked the peripheral and central hyperactivity induced by (+)-MDMA to levels observed in saline control rats. In contrast, WAY 100635 was ineffective, even at the relatively high doses used (Forster et al., 1995). Because GR 127935 possesses high affinity for both 5-HT_{1B} ($pK_i = 8.5$ in rat striatum) and 5-HT_{1D} receptors ($pK_i = 8.5$ –8.7 in guinea pig striatum; Starkey and Skingle, 1994) either or both receptors may contribute to the observed effects. However, few 5-HT_{1D} receptors are localized in rat brain (Bruinvels et al., 1993), and in mice that lack the 5-HT_{1B} receptor protein, MDMA evokes an attenuated hyperactivity compared with wild-type mice (Hen et al., 1998). Thus, the effects of GR 127935 may be

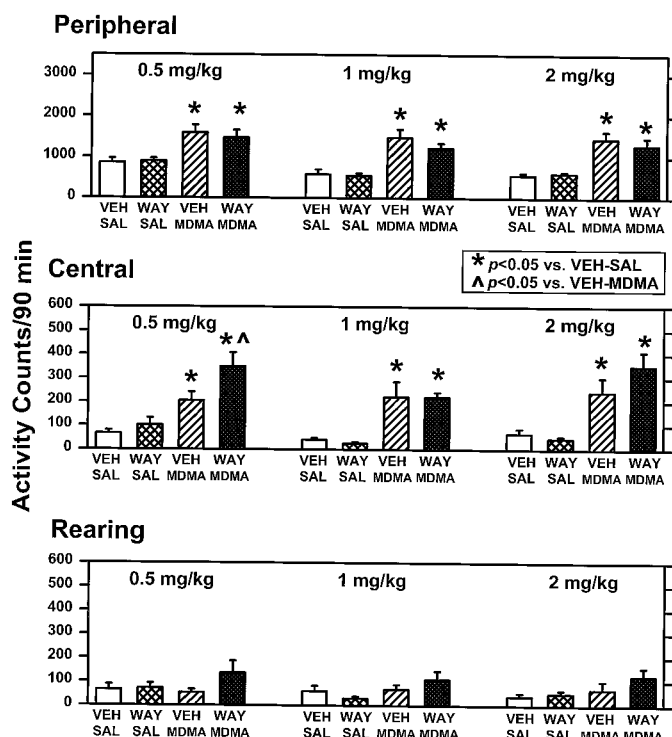


Fig. 3. Effects of acute administration of WAY 100635 (0.5 mg/kg) on spontaneous and (+)-MDMA-induced locomotor activity. Thirty minutes after placement in the activity monitors, rats were injected with WAY 100635 (WAY; 0.5, 1, or 2 mg/kg s.c.) or saline (SAL; 1 ml/kg s.c.) followed 30 min later by (+)-MDMA (MDMA; 3 mg/kg s.c.) or saline (SAL; 1 ml/kg s.c.). Recording of activity began immediately in 5-min time epochs for 90 min. Left, the mean activity counts ($\pm$ S.E.M.) for peripheral (top), central (middle), and rearing (bottom) activity summed over the 90-min period beginning immediately after the second injection (SAL or MDMA; $n = 8$ /group) are shown for the four pretreatment groups: SAL + SAL (open columns), WAY + SAL (cross-hatched columns), SAL + MDMA (left-hatched columns), and WAY + MDMA (dotted columns). *, activity levels that differed significantly from those in SAL + SAL rats. ^, activity levels in WAY + MDMA rats that differed from those in SAL + MDMA rats ($p < .05$).

dependent predominantly on blockade of 5-HT_{1B}, rather than 5-HT_{1D}, receptors.

The 5-HT_{1B/1D} heteroreceptors are found in abundance in circuits that play a major role in psychostimulant-induced motor activity (Bruinvels et al., 1993), such as the mesoaccumbens DA pathway. Presumed activation of 5-HT_{1B} receptors in the ventral tegmental area (VTA) after microinjection of RU 24969 resulted in a long-lasting hyperlocomotion and potentiation of hyperactivity evoked by systemic administration of amphetamine (Oberlander, 1983). Stimulation of 5-HT_{1B/1D} receptors has been shown to depress evoked γ -aminobutyric acid-mediated inhibitory synaptic input to DA neurons of the substantia nigra in vitro (Johnson et al., 1992), an effect that could result in disinhibition of DA neurons. Although knowledge of the manner in which MDMA interacts with VTA DA neurons is limited (Gifford et al., 1996), antagonism by GR 127935 of 5-HT_{1B/1D} receptors in the VTA that control the output of DA neurons may contribute to the GR 127935-evoked blockade of MDMA-induced hyperactivity.

Administration of MDMA has also been shown to increase DA efflux in striatal regions in vivo (Yamamoto et al., 1995); the time course of MDMA-induced increases in extracellular DA levels is delayed (White et al., 1994; Yamamoto et al., 1995) compared with the immediate increases in 5-HT efflux

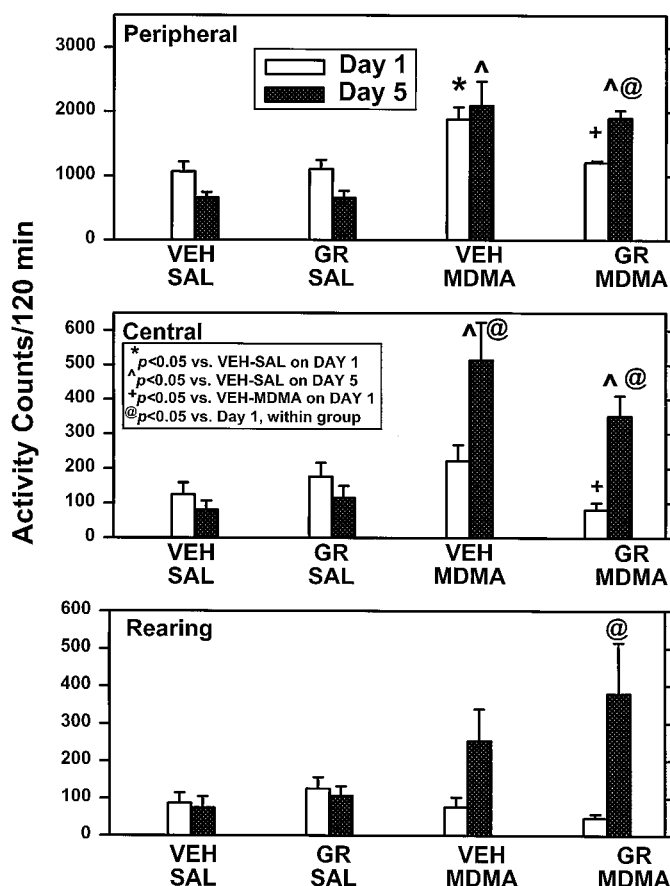


Fig. 4. Effects of pretreatment with GR 127935 (2.5 mg/kg) on spontaneous and (+)-MDMA-induced activity: day 1 and day 5. After a 15-min habituation on each test day, rats were pretreated with GR 127935 (GR; 2.5 mg/kg s.c.) or vehicle 45 min before an injection of (+)-MDMA (MDMA; 3 mg/kg s.c.) or saline (SAL; 1 ml/kg s.c.). The mean activity counts ($\pm$ S.E.M.) summed over the 120-min period beginning immediately after the second injection (SAL or MDMA; $n = 8$ /group) are shown for day 1 (open columns) and day 5 (dotted columns) for the four pretreatment groups: VEH + SAL, GR + SAL, VEH + MDMA, and GR + MDMA. *, activity levels that differed significantly from those in VEH + SAL rats on day 1 of pretreatment. ^, activity levels that differed significantly from those in VEH + SAL rats on day 5. +, activity levels that differed significantly from those in VEH + MDMA rats on day 1. @, activity levels within the same treatment group that differed significantly from day 1 to day 5.

seen (White et al., 1994). The indirectly mediated release of DA may contribute to the inhibition of glutamate-stimulated firing of nucleus accumbens (NAc) neurons evoked by MDMA (Obradovic et al., 1996). A specific role for 5-HT_{1B/1D} receptors in 5-HT-mediated DA release is supported by the findings that coprefusion with GR 127935 blocked 5-HT-mediated DA efflux in rat frontal cortex in vivo (Iyer and Bradberry, 1996), whereas systemic administration of RU 24969 increased DA efflux in the NAc shell (Boulenguez et al., 1996). Thus, the 5-HT_{1B/1D} antagonist GR 127935 may block MDMA-induced hyperactivity by antagonizing the 5-HT_{1B/1D}-mediated events that transpire in striatal and/or limbic regions to result in enhanced, impulse-dependent DA efflux.

Enhanced DA efflux induced by MDMA may be mediated by increased extracellular 5-HT acting at 5-HT₂ receptors to (directly or indirectly) suppress γ -aminobutyric acid release and result in disinhibition of DA neurons and enhancement of DA release (Yamamoto et al., 1995). Isomers of MDMA

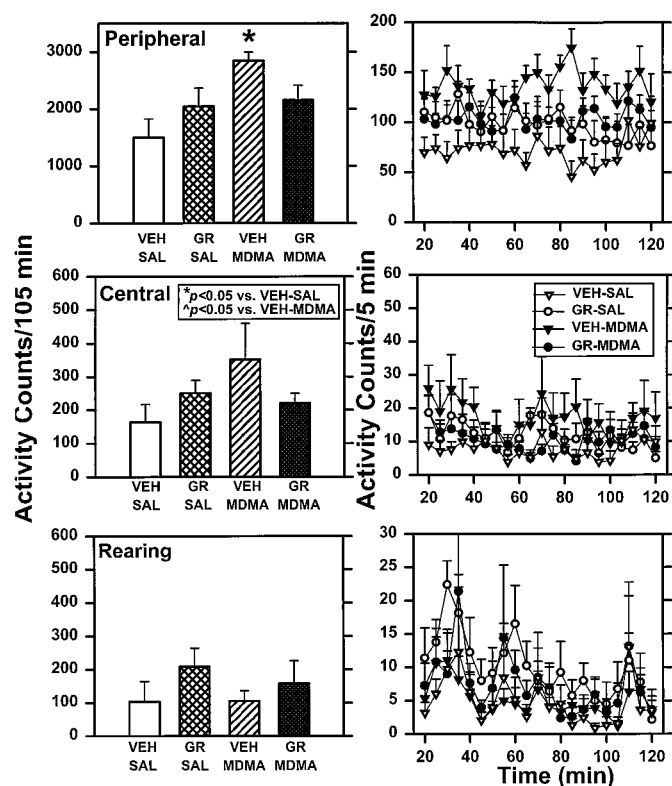


Fig. 5. Challenge test with RU 24969 (2 mg/kg) during withdrawal. Forty-eight hours after the last pretreatment injection, rats were re-habituated to the test environment for 60 min and subsequently challenged with an injection of RU 24969 (2 mg/kg i.p.). Activity was recorded in 5-min time epochs for 105 min beginning 15 min after RU 24969 injection. Left, the mean activity counts ($\pm$ S.E.M.) summed over the 105-min period beginning 15 min after the injection of RU 24969 are shown for the four pretreatment groups: VEH + SAL (open columns), GR + SAL (cross-hatched columns), VEH + MDMA (left-hatched columns), and GR + MDMA (dotted columns). *, activity levels that differed significantly from those in VEH + SAL rats. Right, data are presented as mean activity counts ($\pm$ S.E.M.) in each 5-min bin over the 105-min period beginning 15 min after the RU 24969 injection for the VEH + SAL (∇), GR + SAL ($\circ$), VEH + MDMA ($\blacktriangledown$), and GR + MDMA ($\bullet$) rats.

TABLE 3

5-HT and 5-HIAA content and turnover

Rats were pretreated with GR 127935 (2.5 mg/kg) or β -cyclodextrin vehicle (2 ml/kg) 45 min prior to an injection of (+)-MDMA (3 mg/kg) or saline (1 ml/kg). After a 48-h withdrawal, the hyperactivity evoked by challenge with RU 24969 (2 mg/kg) was assessed. Twenty-four hours after the RU 24969 challenge, rats were anesthetized with chloral hydrate (800 mg/kg i.p.) and decapitated. The frontal cortex was dissected and the tissue was stored at -80°C until assay.

	5-HT	5-HIAA	5-HT/5-HIAA
	ng/mg protein		
Vehicle + saline	26.78 $\pm$ 1.92	1.76 $\pm$ 0.14	15.46 $\pm$ 1.08
GR 127935 + saline	28.35 $\pm$ 1.16	1.68 $\pm$ 0.16	17.51 $\pm$ 1.09
Vehicle + (+)-MDMA	27.11 $\pm$ 1.71	1.54 $\pm$ 0.09	17.88 $\pm$ 1.20
GR 127935 + (+)-MDMA	30.78 $\pm$ 2.66	1.79 $\pm$ 0.15	17.49 $\pm$ 1.28

exhibit affinity for 5-HT_{2A} receptors in the low micromolar range, and a single high dose of MDMA can result in brain concentrations that could trigger direct stimulation of 5-HT_{2A} receptors (Battaglia et al., 1988). GR 127935 exhibits affinity for 5-HT_{2A} receptors (pK_i = 7.4; Skingle et al., 1996), and moderate 5-HT_{2A} antagonist activity has been reported for this drug (De Vries et al., 1997). However, the hyperactivity induced by a low dose of (+)-MDMA has been found to be insensitive to 5-HT₂ antagonists (M. G. Bankson and K. A. Cunningham, unpublished observation; Callaway et al.,

1991), whereas hyperactivity induced by a high dose (20 mg/kg) of ($\pm$)-MDMA was attenuated by antagonists with affinity for 5-HT_{1B/1D} and 5-HT_{2A/2C} receptors, and even DA receptors (Kehne et al., 1996). Perhaps at high doses of MDMA, the probability of 5-HT_{2A} stimulation increases because either released 5-HT or MDMA itself could bind to 5-HT_{2A} receptors. At lower doses, a preferential stimulation of 5-HT_{1B/1D} receptors by released 5-HT itself may occur because the affinity of 5-HT is higher for 5-HT_{1B/1D} receptors than for 5-HT₂ receptors (Zifa and Fillion, 1992).

Hyperactivity Induced by Chronic (+)-MDMA: Role of 5-HT_{1B/1D} Receptors. Sensitization to MDMA administered at non-neurotoxic doses has been demonstrated in other studies, although tolerance and no effect of repeated MDMA treatment have also been reported (see introduction). In the present experiment, repeated (+)-MDMA administration resulted in sensitization of central activity only, whereas rats pretreated with GR 127935 + (+)-MDMA exhibited augmentation of peripheral and central activity despite the effectiveness of GR 127935 (2.5 mg/kg) to suppress (+)-MDMA-induced hyperactivity on day 1 of the chronic regimen. These data suggest that, at least at the dose and regimen used here, the development of a progressive increase in activity after repeated (+)-MDMA exposure was not hampered by antagonism of 5-HT_{1B/1D} receptors. In fact, these data might be interpreted as an augmentation of (+)-MDMA sensitization development because both peripheral and central activity increased from days 1 to 5 of repeated GR 127935 + (+)-MDMA treatment. However, this conclusion is not supported by the fact that maximal activity achieved in the GR 127935 + (+)-MDMA rats was not higher than that observed in vehicle + (+)-MDMA rats on day 5. Furthermore, because (+)-MDMA-evoked hyperactivity was significantly attenuated by GR 127935 on day 1, activity levels between days 1 and 5 would be more likely to differ for the rats treated with GR 127935-(+)-MDMA than for the rats pretreated with (+)-MDMA alone. Thus, although 5-HT_{1B/1D} receptors are an important mediator of the acute hyperactivity evoked by a low dose of (+)-MDMA, these receptors may play only a tangential role in the development of sensitization to (+)-MDMA. Alternatively, a complex interplay between 5-HT_{1B/1D} receptors and DA function exists (see above), and with repeated (+)-MDMA exposure, the actions of MDMA to evoke hyperactivity may become less dependent on 5-HT_{1B/1D} neurotransmission and more reliant on DA mechanisms. In fact, MDMA-evoked extracellular DA efflux in the NAc was augmented in MDMA-sensitized rats, similar to what has been shown for amphetamine and cocaine (Kalivas et al., 1998). This neuroplasticity of DA systems with repeated MDMA exposure may not occur independent of 5-HT systems; however, the exact contribution of 5-HT in these changes is difficult to ascertain.

As one means to begin assessing the functional adaptability of 5-HT_{1B} receptor mechanisms after chronic exposure to (+)-MDMA, the hyperactivity induced by the 5-HT_{1B/1A} agonist RU 24969 (Callaway and Geyer, 1992) was established during withdrawal from the chronic regimen. Peripheral activity was significantly augmented after a dose of RU 24969 in (+)-MDMA-treated rats compared with saline-treated controls; a trend toward increased central activity was also evident. The enhanced effect of RU 24969 tentatively supports the hypothesis that (+)-MDMA sensitization is associated

with modification of the receptors that mediate the behavioral effects of RU 24969. Because RU 24969 has actions at 5-HT_{1B} and 5-HT_{1A} receptors that appear to contribute to its effects on locomotion (Kalkman, 1995; O'Neill and Parameswaran, 1997), changes in either or both receptor subtypes could contribute to the enhanced response to RU 24969. However, denervation supersensitivity cannot account for the observed enhancement of RU 24969-induced hyperactivity because the current regimen of (+)-MDMA exposure did not result in any significant alteration of 5-HT, 5-HIAA, or turnover in frontal cortex, a brain region particularly sensitive to the neurotoxic effects of MDMA (Schmidt et al., 1990). Because little is known about alterations in 5-HT receptors after repeated nonneurotoxic doses of MDMA, further studies are needed to elaborate such neuroplastic changes consequent to MDMA sensitization.

Despite the fact that behavioral sensitivity to RU 24969 was unaltered by chronic GR 127935 alone, the supersensitivity to RU 24969 after repeated (+)-MDMA exposure was normalized by cotreatment with GR 127935 because levels of activity expressed by GR 127935 + (+)-MDMA rats in response to RU 24969 were similar to those exhibited by saline-treated rats. Although GR 127935 has a long duration of action (Skingle et al., 1994), preliminary studies in our laboratory do not support an efficacy of this compound to block 5-HT_{1B}-mediated hyperactivity 48 h after GR 127935 injection (our unpublished observation); therefore, the presence of measurable levels of GR 127935 probably does not account for the observed attenuation of the behavioral response to RU 24969 observed 48 h after the last cotreatment injection with GR 127935 + (+)-MDMA. However, this normalization of behavioral responsiveness to RU 24969 occurred in the face of effective sensitization development in rats treated with GR 127935 + (+)-MDMA. These data further support the dissociation between activation of 5-HT_{1B} receptors and the development of sensitization to MDMA. At a minimum, these behavioral observations suggest the importance of quantitative analyses of changes in 5-HT_{1B} and 5-HT_{1A} receptors after chronic exposure to sensitizing, low doses (+)-MDMA.

In summary, actions of MDMA to release 5-HT onto 5-HT_{1B/1D} receptors that control DA release in cell-body (e.g., VTA) and/or axonal projection regions (e.g., NAc) may mediate the hyperactivity induced by acute (+)-MDMA administration. After repeated administration, behavioral sensitization to MDMA developed unimpeded by 5-HT_{1B/1D} receptor antagonism with GR 127935. Despite this, withdrawal from repeated (+)-MDMA treatment was associated with an augmented response to RU 24969-induced hyperactivity, suggesting that supersensitivity of 5-HT_{1B} (and/or 5-HT_{1A}) receptors had developed. These data suggest that a complex interaction between 5-HT_{1B/1D} receptor function and the mechanisms (possibly predominantly dopaminergic) underlying MDMA sensitization exists.

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