Psychopharmacology (1999) 141 : 154–161

ORIGINAL INVESTIGATION

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Locomotor response to MDMA is attenuated in knockout mice lacking the 5-HT_{1B} receptor

Received: 5 February 1998 / Final version: 7 May 1998

Abstract 3,4-Methylenedioxymethamphetamine (MDMA) is a psychoactive drug of abuse which is increasingly popular in human recreational drug use. In rats, the drug has been shown to stimulate locomotion while decreasing exploratory behavior. MDMA acts as an indirect agonist of serotonin (5-HT) receptors by inducing 5-HT release by a 5-HT reuptake transporter-dependent mechanism, although it is not known which 5-HT receptors are important for the behavioral effects of the drug. In order to examine the role of specific 5-HT receptors, we assessed the behavioral effects of MDMA on knockout mice lacking the 5-HT_{1B} receptor. Knockout animals show a reduced locomotor response to MDMA, although delayed locomotor stimulation is present in these animals. This finding indicates that the locomotor effects of MDMA are dependent upon the 5-HT_{1B} receptor, at least in part. In contrast, MDMA eliminates exploratory behavior in both normal and knockout mice, suggesting that the exploratory suppression induced by MDMA occurs through mechanisms other than activation of the 5-HT_{1B} receptor. To confirm these findings, we tested the effects of MDMA on the locomotor and exploratory behavior of wild-type mice pretreated with GR 127935, a 5-$HT_{1B/1D}$ receptor antagonist. These mice had an attenuated locomotor response to MDMA, but still exhibited the drug-induced suppression of exploration.

Key words 3,4-Methylenedioxymethamphetamine (MDMA) · Serotonin · Locomotion · Exploratory behavior · Knockout mice · Drug abuse · 5-HT_{1B} receptor

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Introduction

MDMA (3,4-methylenedioxy-*N*-methamphetamine, commonly known as Ecstasy) is an increasingly popular drug of abuse in humans, eliciting increased feelings of empathy and affinity. It also has been implicated in serotonin (5-HT) neurotoxicity (Battaglia et al. 1991). In laboratory animals, it increases locomotion, decreases exploratory behavior, disrupts startle plasticity, and disrupts schedule-controlled responding (Glennon and Young 1984; Gold et al. 1988; Li et al. 1989; Paulus and Geyer 1992; Dulawa et al. 1997). The neural substrates underlying these behavioral effects are poorly understood.

Like many drugs of abuse, MDMA acts at several neural targets. It causes the release of 5-HT and dopamine (DA) via the reuptake transporters (Schmidt et al. 1987). MDMA therefore acts as an indirect agonist of all 5-HT and DA receptor subtypes. Although the locomotor effects of other drugs of abuse, including cocaine, primarily depend on the DA system (Wise and Bozarth 1987), the 5-HT system appears to be essential in mediating the effects of MDMA. Fluoxetine, a specific inhibitor of the 5-HT reuptake transporter, can block the 5-HT releasing effects of MDMA (Hekmatpanah and Peroutka 1990; Berger et al. 1992) and also reduces striatal DA release (Gudelsky and Nash 1996). Moreover, fluoxetine pretreatment antagonizes MDMA-induced locomotor hyperactivity in rats (Callaway et al. 1990).

While release of 5-HT appears to be necessary to evoke the locomotor stimulating effects of MDMA, it is unclear which 5-HT receptors mediate these effects. Indirect evidence has suggested that activation of the 5-HT_{1B} receptor may be crucial. The behavioral effects of the 5-$HT_{1B/1A}$ agonist RU 24969 are very similar to those of MDMA. In rodents, both drugs elicit increased locomotion, decreased exploratory rearings and hole pokes, and a straight-line pattern of locomotion with low variability in the pattern of movements

(Rempel et al. 1993). In contrast, specific 5-HT_{1A} and 5-$HT_{2A/2C}$ agonists decrease both locomotion and exploratory behaviors (Mittman and Geyer 1989; Wing et al. 1990). Repeated administrations of RU 24969, but not 5-HT_{1A} or 5-$HT_{2A/2C}$ agonists, reduce the behavioral response to MDMA, indicating behavioral cross-tolerance (Callaway and Geyer 1992). Furthermore, both propanolol and pindolol, β-adrenergic antagonists with affinity for 5-HT_1 receptors, are able to antagonize MDMA-induced hyperactivity (Callaway et al. 1992; Rempel et al. 1993). These studies indicate that 5-HT_1 receptors, particularly 5-HT_{1B} receptors, are good candidates for the mediation of locomotor activation produced by serotonin releasers like MDMA.

It has been difficult to assess directly the role of the 5-HT_{1B} receptor in mediating the effects of drugs of abuse because of the lack of specific ligands for this receptor (Hoyer et al. 1994). However, gene-targeting technology has allowed us to create a strain of knockout mice (KO) lacking the gene for the 5-HT_{1B} receptor. We have already demonstrated that these mice display no locomotor response to RU 24969 (Saudou et al. 1994), indicating that RU 24969 stimulates locomotion via the 5-HT_{1B} receptor. Here, we use 5-HT_{1B} KO mice to test the hypothesis that the 5-HT_{1B} receptor is essential for MDMA-induced locomotion. We demonstrate that MDMA-induced locomotor stimulation is dependent on the 5-HT_{1B} receptor, although high doses appear to be able to stimulate locomotion via other neurotransmitter systems. Furthermore, we show that MDMA can decrease exploratory behavior independently of the 5-HT_{1B} receptor.

Materials and methods

The wild-type (WT) and KO mice used in these experiments have a pure 129/Sv genetic background. They were bred and raised in our facility, on a 12-h light-dark cycle (6 a.m. to 6 p.m.). Animals were housed four to five per cage, with freely available food and water. Principles of laboratory animal care (NIH publication No. 85–23, revised 1985) were followed. Drug-naive male mice 4–6 months old and weighing 22–35 g were used.

MDMA (3,4-methylenedioxy-*N*-methamphetamine HCl, Sigma Pharmaceutical) was dissolved in sterile 0.9% saline solution on the day of testing. GR 127935 (Glaxo Wellcome) was dissolved in sterile distilled water by gently heating the solution for 20 min. All injections were given intraperitoneally (IP) in a volume of 0.2 ml (6.6 ml/kg).

Testing was conducted between 7 a.m. and 5 p.m. Animals were placed in 40 cm by 40 cm square, open field chambers. They were monitored throughout the test session by a video-tracking system (PolyTrack, San Diego Instruments, San Diego, Calif., USA) that monitors up to four animals simultaneously and records each animal's position every 0.5 s. The system is also equipped with infrared photobeams located 4 cm above the floor of the open field that record a rearing event whenever the animal rears. Similarly, eight nose-poke holes located around the perimeter of the field record a nose-poke event whenever an animal investigates a hole. Animals were videotaped throughout all test sessions for later evaluation of stereotyped behavior.

In order to habituate animals to the testing procedure, on the day before testing began, mice were given IP saline injections and then monitored in the open field for 30 min. For drug testing, animals were brought to the testing room 1 h before the test session began. Animals were treated with 3.3 mg/kg (n = 8 WT and 8 KO), 10 mg/kg (n = 10 WT and 10 KO), or 30 mg/kg (n = 8 WT and 8 KO) of MDMA or saline vehicle (n = 17 WT and 19 KO) 10 min before the test session. Then, animals were placed directly in the open field and monitored continuously for 90 min. Data regarding each animal's path length, rearing and nose poke behavior, and the time spent in the center of the open field was collected and summed for each 5-min interval during the test session. These successive measurements were analyzed using two-factor repeated measures analysis of variance (ANOVA), with dose and genotype as factors and behavior during each 5-min interval as the repeated measure. Post-hoc Scheffé comparisons were used where necessary.

Two trained observers who were blind to both mouse genotype and drug treatment watched videotapes of test sessions and evaluated stereotyped behavior. Each animal was observed for 1 min at seven different time points during the test session: 0, 15, 30, 45, 60, 75 and 90 min after testing began. Each mouse was assigned a single numerical score for each time period: 0: normal, quiet behavior; 1: normal, exploratory behavior; 2: rapid locomotion; 3: repetitive movement in a restricted area of the cage; 4: intense stereotypy (circling and head weaving) in one area of the cage (Tolliver and Carney 1994). The number of time points during which each animal engaged in each of these behaviors was summed and analyzed using two-factor ANOVA and post-hoc Scheffé comparisons.

For the antagonist experiment, WT mice were treated with a 10 mg/kg dose of GR 127935 (n = 9) or vehicle (n = 10) 30 min before the injection of 30 mg/kg MDMA. A third control group (n = 10) was given two vehicle injections instead of GR 127935 or MDMA. In all other respects, the protocol and data analysis exactly matched the experiments conducted with MDMA alone.

Results

The responses of KO and WT mice to 3.3, 10 and 30 mg/kg MDMA are shown in Fig. 1. The lowest dose (3.3 mg/kg) failed to stimulate locomotion in either genotype. For WT mice, 10 mg/kg nearly doubled baseline locomotion levels, while 30 mg/kg increased locomotion four-fold (Fig. 1a). There was a main effect of Treatment ($F_{3,39}$ = 11.66, $P < 0.0001$), and an interaction of Treatment by Time ($F_{51,663}$ = 1.58, $P < 0.01$). In KO mice, only 30 mg/kg obviously stimulated locomotion, and the peak response was reached much later than for WT mice (70 min versus. 25 min after testing began; Fig. 1b). The KO showed a main effect of Treatment ($F_{3,40}$ = 12.43, $P < 0.0001$), a main effect of Time ($F_{17,680}$ = 8.27, $P < 0.0001$), and an interaction of Treatment by Time ($F_{51,680}$ = 12.03, $P < 0.0001$). Because of the clear difference in the time course of the effects of 30 mg/kg MDMA for WT and KO mice, we chose to analyze the initial 30 min of testing and the final 30 min separately. This analysis revealed interesting distinctions, suggesting that there are two or more qualitatively distinct phases of the effects of MDMA. In the initial 30 min, the effects of MDMA differed strongly between the two genotypes (Fig. 1c). There was a main effect of Genotype ($F_{1,85}$ = 5.22, $P < 0.05$), a main effect of Treatment

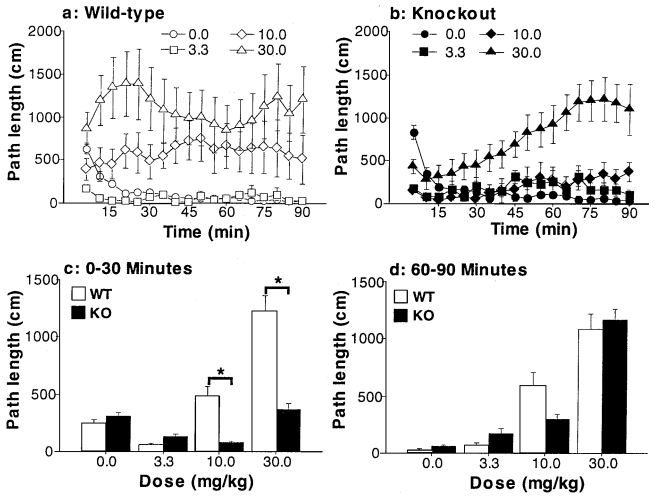

Fig. 1 **a** Mean path length (in cm) ± SEM for WT males treated with saline ($n = 17$), 3.3 mg/kg ($n = 8$), 10 mg/kg ($n = 10$) and 30 mg/kg ($n = 8$) MDMA. **b** Mean path length ± SEM for KO males treated with saline ($n = 19$), 3.3 mg/kg ($n = 8$), 10 mg/kg ($n = 10$) and 30 mg/kg ($n = 8$). **c,d** Mean path length ± SEM during the first 30 min and last 30 min of the test session, respectively. *Columns* are the average path length per 5-min interval. Significant differences between genotypes ($P < 0.05$) are marked with *

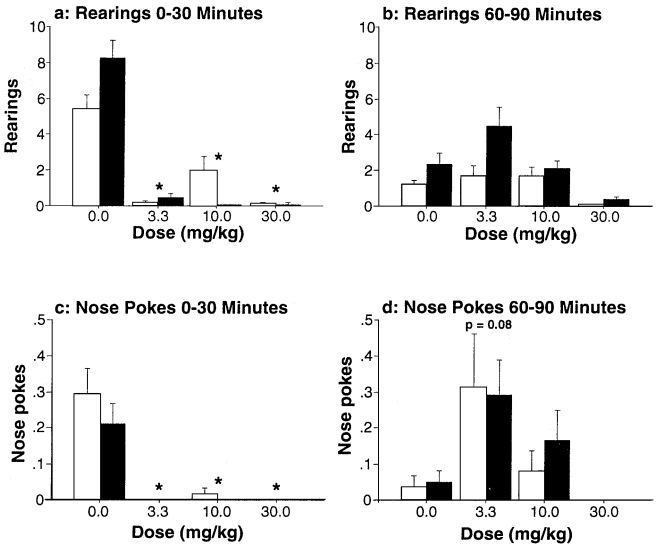

Fig. 2 **a,b** Number of rearings per 5 min ± SEM averaged during the first 30 and last 30 min of testing. **c,d** Number of nose pokes per 5 min ± SEM averaged during first 30 and last 30 min of testing. The number of animals in each condition is the same as for Fig. 1. Treatments that differ significantly ($P < 0.05$) from saline are marked with *. □ WT, ■ KO

($F_{3,83} = 10.03$, $P < 0.0001$), a main effect of Time ($F_{5,415} = 13.96$, $P < 0.0001$), an interaction of Treatment by Time ($F_{15,415} = 11.66$, $P < 0.0001$), and an interaction of Genotype by Time ($F_{5,425} = 3.10$, $P < 0.01$). Post-hoc comparisons showed significant differences between WT and KO mice treated with 10 mg/kg ($P < 0.05$) and 30 mg/kg ($P < 0.05$). In the last 30 min (Fig. 1d), in contrast, there were no significant genotype differences or interactions, although there was still a main effect of Treatment ($F_{3,83} = 20.39$, $P < 0.0001$) and an interaction of Treatment by Time ($F_{14,415} = 1.94$, $P < 0.05$). In summary, both 10 and 30 mg/kg MDMA stimulated locomotion in WT but not in KO mice during the first 30 min of the test session. By the end of the test, however, 30 mg/kg MDMA stimulated locomotion to the same extent in WT and KO mice.

Since MDMA has been shown to reduce exploratory activity (rearings and nose pokes) in rats concurrently with its locomotor activating effects (Gold et al. 1988), we examined exploratory behaviors in WT and KO mice in response to the drug. In accordance with our locomotor analysis, we analyzed the first 30 min and the final 30 min separately. Early in the test, MDMA nearly eliminated rearings in both genotypes (Fig. 2a). There was a main effect of Treatment ($F_{3,84} = 12.94$, $P < 0.0001$), a main effect of Time ($F_{5,420} = 13.69$, $P < 0.0001$), and an interaction of Treatment by Time ($F_{15,420} = 8.55$, $P < 0.0001$), but there were no effects or interactions of genotype. Later in the test, this suppression of rearings was no longer apparent except in animals treated with 30 mg/kg MDMA (Fig. 2b). There were no effects of Genotype or Treatment, although there was still a main effect of Time ($F_{6,504} = 2.38$, $P < 0.05$). Nose pokes, another measure of exploratory behavior, were almost completely eliminated by MDMA in both genotypes during the first 30 min of the test (Fig. 2c). There was a main effect of treatment ($F_{3,84} = 9.08$, $P < 0.0001$). No significant effects or interactions of genotype or time were seen. In the final 30 min, 30 mg/kg MDMA still blocked all nose poke behavior in both genotypes (Fig. 2d). Although 3.3 mg/kg MDMA appears to increase nose pokes slightly, this effect was not significantly different from saline ($P = 0.08$). Overall, there was a main effect of Treatment ($F_{3,84} = 2.97$, $P < 0.05$), but no effects of genotype or time. In summary, MDMA suppressed exploratory behavior, as measured by rearings and nose pokes, equally in both KO and WT mice.

Previous analyses of the qualitative characteristics of the locomotion induced by MDMA treatment have indicated that it causes very stereotyped, focused straight-line locomotion (Gold et al. 1988). This type of locomotion usually results in a characteristic pattern in which the animal runs in straight lines around the periphery of the open field, rarely varying its path or entering the center of the open field (Gold et al. 1988; Paulus et al. 1990). Indeed, the WT mice showed characteristic paths that appeared to be composed of straight-line ambulation around the edges of the open field. The paths of KO mice, in contrast, had more turns and curves and used more of the open field. To qualitatively confirm this observation, we examined the amount of time spent in the center of the open field. Other psychostimulants that increase locomotion, such as cocaine and amphetamine, increase the amount of

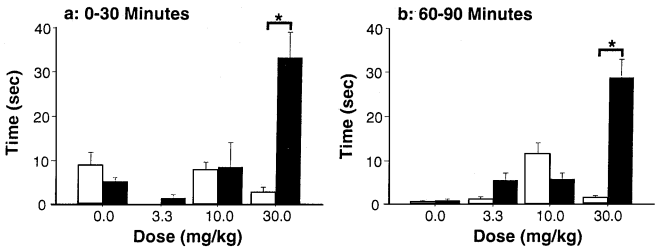

Fig. 3a,b Mean number of seconds (±SEM) spent in center of the open field in each 5-min interval during the first 30 or last 30 min of testing. The number of animals in each condition is the same as for Fig. 1. Significant differences between genotypes ($P < 0.05$) are marked with *. □ WT, ■ KO

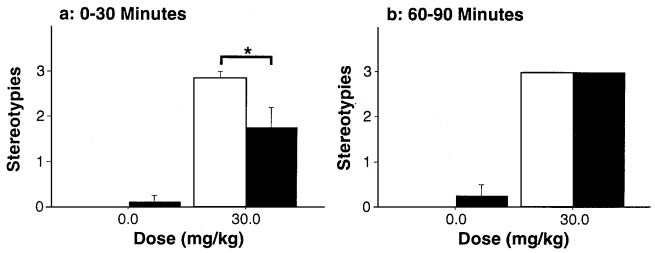

Fig. 4a,b The number of times, out of three observations, that an animal was scored as exhibiting stereotyped behavior during either the first 30 min (**a**) or last 30 min (**b**) of the test session. Scores from ten saline-treated animals of each genotype and eight MDMA-treated animals of each genotype are shown. Significant differences between genotypes ($P < 0.05$) are marked with *. □ WT, ■ KO

time spent in the center (our unpublished observations). While the highest dose of MDMA did increase the time KO mice spent in the center to over 30 s in each 300-s interval, WT mice given the same dose spent less than 5 s in the center. In the first 30 min (Fig. 3a), there was a main effect of Treatment ($F_{3,84} = 3.34$, $P < 0.05$), a main effect of Time ($F_{5,420} = 3.12$, $P < 0.01$) and an interaction of Genotype by Time ($F_{5,430} = 2.41$, $P < 0.05$). Post-hoc comparisons showed a significant difference between WT and KO mice treated with 30 mg/kg ($P < 0.005$). In the final 30 min (Fig. 3b), a similar pattern was apparent, with a main effect of Treatment ($F_{3,84} = 5.61$, $P < 0.005$) and a genotype difference at 30 mg/kg ($P < 0.01$). To confirm the validity of this measure, we also calculated the percentage of locomotion that occurred in the center (path length in center/total path length). This measure yielded an identical pattern of results (not shown).

There is often competition between the expression of horizontal locomotion versus stereotyped behavior. Thus, it is possible that the decreased locomotion in the KO can be explained by KO mice engaging in more stereotyped behavior. To investigate this possibility, trained observers assessed the degree of stereotyped behavior exhibited by animals in these experiments. Animals treated with 3.3 or 10 mg/kg MDMA exhibited no stereotypy. However, WT and KO animals treated with 30 mg/kg almost all showed either repetitive movement or intense, focused stereotypy. In the first 30 min, WT mice showed significantly more stereotyped behavior (Fig. 4a). There was a main effect of Treatment ($F_{1,28} = 85.59$, $P < 0.0001$), a main effect of Genotype ($F_{1,28} = 4.23$, $P < 0.05$), and an interaction of Treatment by Genotype ($F_{1,28} = 6.60$, $P < 0.05$). In the last 30 min, however, all WT and KO mice treated with 30 mg/kg exhibited abnormal behavior at all times (Fig. 4b). Since the degree of stereotyped behavior in KO mice was less than or equal to WT at all times, their decreased locomotor response cannot be attributed to an increase in stereotypy.

In order to confirm that the KO's attenuated response to MDMA is a direct result of the absence of the 5-HT_{1B} receptor, we pretreated the WT mice with 10 mg/kg GR 127935, an antagonist of the 5-$HT_{1B/1D}$

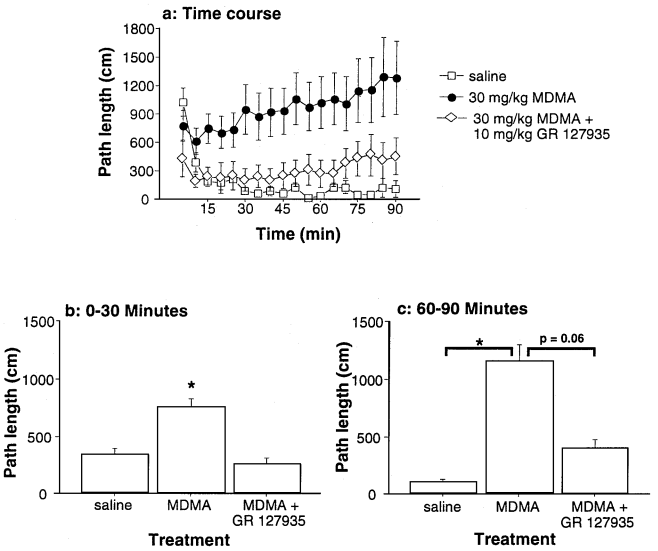

Fig. 5 (**a**) Mean path length for WT mice treated with saline ($n = 10$), 30 mg/kg MDMA ($n = 10$), or 10 mg/kg GR 127935 followed by 30 mg/kg MDMA ($n = 9$). *Lower panels* show mean path length ± SEM during the first 30 min (**b**) and last 30 min (**c**) of the test session. *Columns* are the average path length per 5-min interval. Treatments that differ significantly ($P < 0.05$) from saline are marked with *

receptor (Skingle et al. 1993), and compared their response to 30 mg/kg MDMA with that of WT treated with MDMA alone. We have previously shown that 10 mg/kg GR 127935 can attenuate the locomotor effects of cocaine in WT mice, but when administered alone, it has no effect on the locomotor or exploratory behavior of either WT or KO mice (Castanon et al. 1996). In this experiment, GR 127935 blocked all of the locomotor stimulation caused by MDMA during the first 30 min of the test (Fig. 5b). In the first 30 min, there was a main effect of Treatment ($F_{2,25} = 6.01$, $P < 0.01$), a main effect of Time ($F_{5,125} = 7.45$, $P < 0.0001$), and an interaction of Treatment by Time ($F_{10,125} = 4.47$, $P < 0.0001$). Post-hoc comparisons showed significant differences between saline and MDMA alone ($P < 0.04$) and between GR 127935

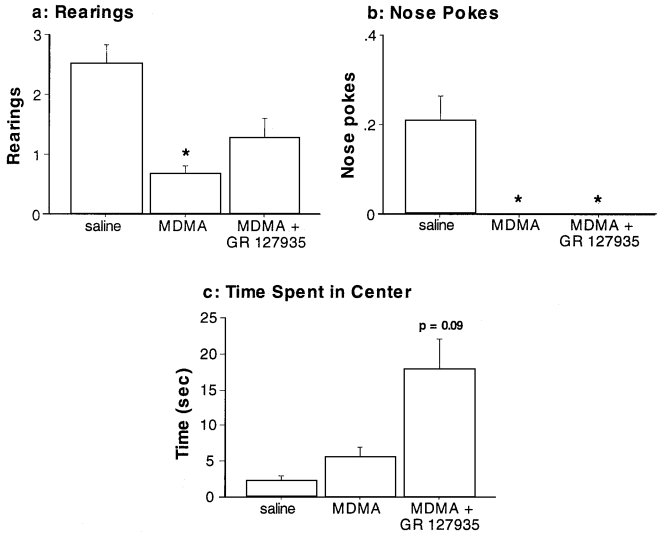

Fig. 6 **a** Mean number of rearings ± SEM averaged for each 5-min interval for WT mice treated with saline (n = 10), 30 mg/kg MDMA ($n = 10$), or 10 mg/kg GR 127935 followed by 30 mg/kg MDMA ($n = 9$). **b** Mean number of nose pokes during the same intervals. **c** Mean number of seconds spent in the center of the open field. Treatments that differ significantly ($P < 0.05$) from saline are marked with *

pretreatment and MDMA alone ($P < 0.05$). In the last 30 min, there was a main effect of MDMA Treatment ($F_{2,25} = 6.64$, $P < 0.005$), but no effect of Time and no interactions (Fig. 5c). Post-hoc comparisons showed a significant difference between MDMA and saline-treated animals ($P < 0.01$), but the difference between animals pretreated with GR 127935 and animals given MDMA alone did not reach significance ($P = 0.06$). Thus, WT mice treated with MDMA and GR 127935 had a similar pattern and time course of locomotor activation when compared to KO mice treated with MDMA alone.

We also assessed the effect of a 5-$HT_{1B/1D}$ antagonist on MDMA-induced suppression of exploration. GR 127935, the 5-$HT_{1B/1D}$ antagonist, did not protect against MDMA-induced reductions in exploratory behavior (Fig. 6). Rearings were reduced in mice treated with MDMA alone relative to control animals (Fig. 6a). There was a main effect of treatment ($F_{2,24} = 5.37$, $P < 0.05$), a main effect of time ($F_{17,408} = 5.45$, $P < 0.0001$), and an interaction of Treatment by Time ($F_{34,408} = 2.81$, $P < 0.0001$). Post hoc comparisons showed that the MDMA group was significantly lower than saline controls ($P < 0.05$), while the MDMA plus GR 127935 did not differ from saline. Treatment with MDMA completely eliminated nose pokes, regardless of whether the animal was pretreated with GR 127935 or not (Fig. 6b). There was a main effect of Treatment ($F_{2,24} = 11.91$, $P < 0.0005$) with no effects of Time. Mice treated with MDMA alone ($P < 0.005$) and MDMA plus GR 127935 ($P < 0.005$) had significantly fewer nose pokes than saline controls. Thus, blockade of the 5-HT_{1B} receptor cannot antagonize the effect of MDMA on exploratory behavior. We also assessed time spent in the center of the open field, to see if WT mice pretreated with GR 127935 showed an increase in time in center similar to KO mice (Fig. 6c). Although these mice did show more time in the center (about 20 s in each 300-s interval, compared with 5 s for those treated with MDMA alone), this difference was not significant ($P = 0.09$).

Discussion

This study provides evidence that the locomotor stimulating effects of the 5-HT releaser MDMA are mediated by the 5-HT_{1B} receptor, while the reduction in exploration produced by MDMA occurs independently of this receptor. KO mice, which show normal baseline locomotion, showed very little response to 10 mg/kg MDMA, although this dose robustly stimulated locomotion in WT mice. Nevertheless, near the end of the test session, a slight increase in locomotion in KO mice treated with 10 mg/kg MDMA was apparent. It is possible that other receptors or neurotransmitters are recruited in this response, and begin to affect the mouse after about 1 h. This latter explanation seems most likely when we consider the response to 30 mg/kg. This dose immediately stimulated locomotion in WT mice, who reach a peak level of locomotion after only 25 min. They remained near this high level throughout the test session. In KO mice, however, the initial response to 30 mg/kg MDMA was actually a suppression of locomotion relative to saline (in the first 5 min). This high dose eventually caused KO mice to show as much locomotion as WT, but these peak levels were not reached until 75 min after the test began.

MDMA is not specific for 5-HT release. It also has a high affinity for the DA transporter and causes DA release (Schmidt et al. 1987; McKenna et al. 1991; White et al. 1994). It is therefore possible, particularly at high doses, that DA release is an important component of the locomotor response to the drug. We have already shown that indirect DA agonists, including cocaine, amphetamine and methylphenidate, cause increased locomotion in 5-HT_{1B} KO mice relative to WT mice (Scearce et al. 1997). In contrast, DA agonists induce less stereotypy in KO mice than WT (Rocha et al. 1998). We have suggested that developmental compensations favoring the influence of the mesolimbic DA system over the mesostriatal pathway may contribute to these behavioral phenotypes. Indeed, we have found increased levels of D_1 receptor, substance P, and dynorphin in KO striatum, indicating that dopaminergic compensations have occurred in KO mice (Scearce et al. 1997). Perhaps the robust MDMA-induced stimulation of locomotion seen in KO mice at higher doses late in the test is a result of the drug acting on this hyperactive DA system, rather than a result

of the serotonergic effects of the drug. There is some evidence that, in rats, the early subjective effects of MDMA are primarily serotonergic, but the later effects have a dopaminergic component (Schechter 1988). Similarly, studies using microdialysis or HPLC have indicated that the MDMA-induced increase in striatal DA release is delayed relative to the increase in 5-HT release (Yamamoto and Spanos 1988; White et al. 1994). These studies agree well with the delayed time course of increased locomotion seen in our KO mice. It is also possible that the locomotor effects seen in KO mice are due to MDMA acting as an indirect agonist at other 5-HT receptors. However, no known agonists of other 5-HT receptors have been shown to stimulate locomotion.

Regardless of the cause of the late-phase locomotor activation in KO mice, the absence of an initial locomotor response to MDMA in KO mice suggests that the drug exerts its locomotor activating effects via the 5-HT_{1B} receptor, at least initially. To confirm that this effect is a direct result of the receptor knockout, we treated WT mice with 10 mg/kg GR 127935, a 5-$HT_{1B/1D}$ receptor antagonist, before administering 30 mg/kg MDMA. The 5-HT_{1B} antagonist attenuated the locomotor response to MDMA, although the animals showed some recovery of locomotion in the final 30 min of testing: a result that is striking in its resemblance to the pattern of MDMA-induced locomotion seen in the KO. The effect of GR 127935 is likely to be mediated by 5-HT_{1B} rather than 5-HT_{1D} receptors because 5-HT_{1B} is much more abundant in this species, particularly in the basal ganglia (Lucas et al. 1997). The antagonist experiment, when considered alongside the KO data, gives strong evidence that the presence of the 5-HT_{1B} receptor is necessary for the full expression of the locomotor activating effects of MDMA.

In contrast, the effects of MDMA on exploratory activity appear to be completely independent of the 5-HT_{1B} receptor. Specifically, the drug virtually eliminates rearing and nose pokes in both genotypes. These effects are robust, occur at all doses tested, and seem to occur with equal potency in both genotypes. Furthermore, WT mice pretreated with GR 127935 also show a decrease in rearings and an elimination of nose pokes after MDMA treatment. The idea that MDMA can reduce all exploratory behaviors through a 5-HT_{1B}-independent mechanism is supported by examining locomotion during the first 5 min of the test. When confronted with a novel open field environment, a mouse's initial response will primarily reflect exploratory tendencies and anxiety-related behavior, rather than pure psychomotor activation. MDMA has been shown to decrease locomotion relative to saline-treated animals during these first few minutes in the open field (Gold et al. 1988). This effect is probably related to the inhibition of exploratory activity, since suppression occurs during the time period when saline-treated animals most actively explore the open field. Indeed, significant inhibition of locomotion during the first 5 min of testing was apparent in KO mice at all doses, and in the WT at 3.3 mg/kg. WT mice treated with GR 127935 followed by 30 mg/kg MDMA also showed suppression of locomotion during the first 5 min. Taken together, these results suggest that the immediate locomotor suppression effects of MDMA, like the suppression of rearing and nose pokes, occur independently of the 5-HT_{1B} receptor. This fits well with pharmacological studies that show that pretreatment with fluoxetine or other 5-HT uptake inhibitors can reduce the locomotor effects of MDMA in rats, but do not affect the MDMA-induced reduction of exploration (Callaway et al. 1990).

It seems clear that in mice as in rats (Geyer and Callaway 1994), the stimulation of locomotion produced by MDMA depends on the 5-HT_{1B} receptor, while the suppression of exploratory activity does not. However, the role of the 5-HT_{1B} receptor in determining the qualitative characteristics of the locomotion is somewhat less clear. A high dose of MDMA (30 mg/kg) stimulated locomotion almost exclusively around the periphery of the open field in WT mice, suggesting that it caused a highly directed, predictable form of locomotion in which the animal moves in a straight line until an obstacle (in this case, the wall of the open field) is encountered. This observation is consistent with the type of locomotion evoked by either MDMA or the 5-HT_{1B} agonist RU 24969 in rats (Gold et al. 1988; Paulus and Geyer 1992; Rempel et al. 1993). KO mice, however, did not exhibit this extreme preference for the periphery of the open field. Indeed, visual inspection of the path made by a KO mouse given 30 mg/kg MDMA suggests that its locomotion is neither extremely straight nor preferentially peripheral. Since we have found that indirect DA agonists like cocaine tend to increase locomotion in the center of the open field (unpublished observation), the increased time in the center seen in KO mice after MDMA may reflect the dopaminergic, rather than serotonergic effects of the drug. In some situations, a preference for the periphery over the center of an open field is taken as a measure of anxiety, since rodents appear to find open spaces aversive. However, in previous studies with rats, the center-avoiding behavior induced by MDMA was not interpreted as evidence of increased anxiety, because familiarization with the testing environment fails to change the pattern of locomotor activity (Callaway et al. 1991). On the other hand, 5-HT_{1B} agonists have been shown to increase anxiety in rodents (Pellow et al. 1987). Without testing the effect of MDMA on a more direct measure of anxiety, such as ultrasonic vocalization in pups or elevated plus-maze behavior, it is difficult to tell whether the center-avoiding behavior seen in WT, but not KO, mice reflects the 5-HT_{1B} receptor's role in anxiety or its characteristic influence on the pattern of locomotor activation.

As one would expect of a drug that can potentially act at numerous 5-HT and DA receptors, the behavioral effects of MDMA are complicated and likely mediated by several receptor subtypes and neurotransmitter systems. The 5-HT_{1B} receptor appears to be central to mediating the effects of MDMA on locomotion. Our previous research leads us to suggest that the DA system in particular may explain the ability of high doses of MDMA to evoke a late stimulation of locomotion in KO mice. While it appears that the 5-HT_{1B} receptor is not required for MDMA to reduce exploration, it is not yet clear what mechanism does mediate this effect. Previous research suggests that the 5-HT_{1A} receptor may mediate the inhibition of exploratory behavior in rats (Geyer and Callaway 1994), although there are also suggestions that these effects might be mediated by non-serotonergic mechanisms (Callaway et al. 1990). Further research, taking advantage of a new 5-HT_{1A} receptor knockout mouse (Ramboz et al. 1997), as well as emerging inducible and tissue-specific knockout technology, should allow us to explore further the role of other receptors and neurotransmitters in mediating the behavioral effects of MDMA.

Acknowledgements This work was supported by NIDA R01DA09862, a Bristol-Myers Squibb Unrestricted Neuroscience Award and an HHMI Predoctoral Fellowship (KS.). GR 127935 was a generous gift from Glaxo Wellcome. The input and advice of Dr. Mark Geyer, Dr. Valerie Compan, and Stephanie Dulawa are gratefully acknowledged.

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