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3,4-*N*-Methlenedioxymethamphetamine-Induced Hypophagia is Maintained in 5-HT_{1B} Receptor Knockout Mice, but Suppressed by the 5-HT_{2C} Receptor Antagonist RS102221

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3,4-Methylenedioxy-*N*-methamphetamine (MDMA or 'ecstasy') is a psychoactive substance, first described as an appetite suppressant in humans, inducing side effects and even death. MDMA increases serotonin (5-HT) levels, and 5-HT inhibits food intake, but the 5-HT receptors involved in MDMA-induced changes in feeding behavior are unknown. We examined whether a systemic MDMA injection would reduce the physiological drive to eat in starved mice and tested if the inactivation of 5-HT_{1B} or 5-HT_{2C} receptors could restore this response. Our results indicate that in starved mice, MDMA (10 mg/kg) provoked an initial hypophagia for 1 h (−77%) followed by a period of hyperphagia (studied between 1 and 3 h). This biphasic feeding behavior due to MDMA treatment was maintained in 5-HT_{1B} receptor-null mice or in animals treated with the 5-$HT_{1B/1D}$ receptor antagonist GR127935 (3 or 10 mg/kg). In contrast, MDMA-induced hypophagia (for the first 1 h period) was suppressed when combined with the 5-HT_{2C} receptor antagonist RS102221 (2 mg/kg). However, RS102221 did not alter MDMA-induced hyperphagia (for the 1–3 h period) but did exert a stimulant effect, when administered alone, during that period. We have previously shown that MDMA or 5-$HT_{1A/1B}$ receptor agonist RU24969 fails to stimulate locomotor activity in 5-HT_{1B} receptor-null mice. Our present data indicate that the 5-HT_{2C} receptor antagonist RS102221 suppresses MDMA-induced hyperlocomotion. These findings provide the first evidence that the inactivation of 5-HT_{2C} receptors may reduce hypophagia and motor response to MDMA, while a genetic deficit or pharmacological inactivation of 5-HT_{1B} receptors was insufficient to alter the feeding response to MDMA.
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INTRODUCTION

It is well known that serotonin (5-HT) regulates feeding behavior, as shown by the potent anorectic properties of 5-HT releasers, such as fenfluramine. Pharmacological studies combined with knockout strategies have clearly demonstrated that among the 15 5-HT receptor subtypes, the 5-HT_{1B} and 5-HT_{2C} receptors are key elements regulating food intake in mammals. The 5-HT_{1B}, 5-HT_{2A}, and 5-HT_{2C} receptor agonists produce hypophagia (Bendotti and Samanin, 1987; Kennett and Curzon, 1988; Schechter and Simansky, 1988; Macor *et al*, 1990; Aulakh *et al*, 1994; Halford and Blundell, 1996; Lee and Simansky, 1997; Lucas *et al*, 1998; Vickers *et al*, 2001; Lee *et al*, 2004). Conversely, the inactivation of 5-HT_{2C} receptors alters fenfluramine-induced anorexia (Neill and Cooper, 1989; Vickers *et al*, 1999, 2001; Heisler *et al*, 2002). 5-HT_{2C} receptor-null mice, known for being obese (Tecott *et al*, 1995), display reduced sensitivity to fenfluramine (Vickers *et al*, 1999, 2001). The possible roles of 5-HT_{1B} receptors in *d*-fenfluramine-induced anorexia are more complex. Acute treatment with GR127935, a 5-HT_{1B} receptor antagonist, or with cyanopindolol, a 5-$HT_{1A/1B}$ receptor antagonist, does not modify *d*-fenfluramine-induced anorexia in starved rats (Vickers *et al*, 2001). In contrast, 5-HT_{1B} receptor-null mice are less sensitive to *d*-fenfluramine than wild-type mice (Lucas *et al*, 1998).

Among drugs that increase synaptic levels of 5-HT, antidepressants are known clearly to modify food intake, whereas the action of some others such as 3,4-*N*-methylenedioxymethamphetamine (MDMA or 'ecstasy') have not been investigated. This is surprising since MDMA is a widely used recreational drug. MDMA is defined as a 'substrate-type 5-HT releaser' (Rothman and Baumann, 2002) and is an amphetamine-like stimulant. MDMA (10 mg/kg) increases the levels of 5-HT, but can also increase dopamine (DA) levels, especially at higher doses (Colado *et al*, 2004).

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The primary goals of this study were to investigate whether MDMA (10 mg/kg) could counteract starvation-induced eating and whether the 5-HT_{1B} and 5-HT_{2C} receptors were involved in this effect.

MATERIALS AND METHODS

Animals

All experiments were performed on male adult wild-type and 5-HT_{1B} receptor-null mice on a 129/Sv genetic background (see Phillips *et al*, 1999 for a description of the exact genetic background of the mice), aged 4–6 months. The 5-HT_{1B} receptor-null mice were generated as described previously (Saudou *et al*, 1994). Both the wild-type and null mice were all obtained from heterozygous breeding at the transgenic animal facility of the UPR CNRS 5203 in Montpellier (France). The genotype of each mouse was identified using the PCR technique. For the 5-HT_{2C} receptor experimental paradigms, wild-type mice on a 129/Sv (Iffa Credo, France) were used at 4–6 months old. Mice were housed five per cage with food and water available *ad libitum* and maintained in a temperature-controlled environment on a 12-h light/dark cycle with light onset at 06:00. The experiments were carried out in accordance with the Guide for Care and Use of Laboratory Animals established by the National Institutes of Health of the United States of America.

Procedures

In summary, three different sets of experiments were performed using the *feeding paradigm* described in detail below and previously used by Lucas and co-workers (1998). First, the wild-type and 5-HT_{1B} receptor-null mice were treated with a single intraperitoneal (i.p.) injection of saline, MDMA, or 5-HT_{1B} receptor agonist. Second, another group of wild-type mice was similarly injected with NaCl, MDMA, or 5-HT_{1B} receptor antagonist GR127935 either alone or in combination with MDMA. Third, NaCl, MDMA, or 5-HT_{2C} receptor antagonist RS102221 alone or combined with MDMA was administered in the same manner to a naïve group of wild-type mice. Finally, four additional groups of wild-type mice were treated with NaCl, MDMA, or RS102221 either alone or coadministered with MDMA and tested in open field chambers (*Activity* section), as described previously (Compan *et al*, 2003, 2004). In each experimental paradigm, animals were treated with an equivalent volume (3.3 ml/kg) of vehicle via the same route.

Feeding Paradigm Tests

The feeding procedure followed was exactly the same as the one used by Lucas and co-workers for fenfluramine (1998). At 3 days before the experiments, 5-HT_{1B} receptor-null and wild-type mice were housed singly with *ad libitum* access to classic food (pellet form, 16.5% crude proteins, 3.6% crude fat, crude fiber 4.6%, ash 5.2%) and tap water. Animals were then food deprived for 24 h with water given *ad libitum* (09:00 to 21:00), as previously used in mice (Lucas *et al*, 1998) and rats (23 h, Vickers *et al*, 2001). Tap water consumption was not measured. On the testing day, classic food was reintroduced into the trough 10 min after an acute i.p. injection of one of the treatments described above. Food was briefly removed (<20 s) and weighed at 1 and 3 h following the initial reintroduction. First, we tested the effects of MDMA (10 mg/kg) in mice of both genotypes. To make sure that our results did not depend on different experimentalists or laboratory conditions, other groups of mice of both genotypes were also treated with the 5-$HT_{1A/1B}$ receptor agonist RU24969 (5 mg/kg), as used by Lucas and co-workers (1998). In parallel, we assessed for the first time the effects of the selective 5-HT_{1B} receptors agonist CGS12066A (0.5, 1 or 2 mg/kg) in wild-type and null mice. Second, wild-type mice received a 3, 5, or 10 mg/kg dose of the 5-$HT_{1B/1D}$ receptor antagonist GR127935 coadministered with or without MDMA (10 mg/kg). Third, a series of experiments were conducted following the injection of a 1 or 2 mg/kg dose of the selective 5-HT_{2C} receptor antagonist RS102221 that was combined or not to MDMA (10 mg/kg) in wild-type mice.

In each experiment, animals received an i.p. injection of sterile 0.9% NaCl. Food intake and body weight were measured daily using a Sartorius CP32S balance (1 mg precision), which automatically provides the mean body weight of 10 values for each animal in movement.

Pharmacological Treatments

MDMA HCl (10 mg/kg, Sigma), 5-$HT_{1A/1B}$ agonist 5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole (RU24969; generously supplied by Roussel-Uclaf, Romainville, France), 5-HT_{1B} agonist 7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrrolo[1,2-*a*]quinoxaline maleate (CGS12066A; 0.5, 1 and 2 mg/kg, Sigma), 5-$HT_{1B/1D}$ receptor antagonist 2′-methyl-4′-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-methoxy-3-(4-methyl-piperazine-1-yl)-phenyl]amide (GR127935; 3 and 10 mg/kg, generously provided by Glaxo Welcome, Taplow, UK), the selective 5-HT_{2C} receptor antagonist 8-[5-(2,4-dimethoxy-5-(4-trifluoromethylphenylsulfonamido)phenyl-5-oxopentyl)]-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (RS102221, 1 and 2 mg/kg, Tocris) were freshly dissolved in sterile 0.9% NaCl before their i.p. injection, on the day of testing.

Activity

Open-field test. Naïve male mice were tested for 3 h following NaCl, MDMA (10 mg/kg), RS102221 (2 mg/kg dose), or MDMA combined with RS102221. Testing was conducted between 09:00 and 17:00. The open-field test environment is a square chamber with an inside area that measures: $43.2 \times 43.2 \times 30.5\,cm^3$. Mice were placed in the center and monitored with 32 infrared light sources spaced 0.5 in apart (1.25 cm) (MED, Associates, Inc., USA) specifically adapted to record the location, the traveled path length, and vertical counts. Two trained experimentalists, who were unaware of which drug was used, scored the number of behavioral stereotypy exhibited for each mouse, rated using the rating scale described by Marona-Lewicka and Nichols (1994) with some modification. Briefly, flat body posture including stretch–attend posture (the mouse stretches its body forward and backward without locomotion), slow stereotyped head weaving (the number of times

the mice made a slow, side to side or lateral movement), licking, and grooming were determined using the following scoring scale: 1 = 1–4 times, 2 = 5–10 times, 3 = 11–20 times, and 4 = more than 20 times. In addition, grooming and licking were counted for each animal. Such a study was not performed for 5-HT_{1B} receptor-related experimental paradigms because we have previously performed detailed analyses indicating that MDMA (10 mg/kg)-induced locomotion is reduced in 5-HT_{1B} receptor-null mice (Scearce-Levie *et al*, 1999; Compan *et al*, 2003).

Data Analysis

Data were analyzed using Statview 5 (Abacus concept, Berkeley, USA). For each set of experiments (Procedure section), a repeated measures ANOVA was systematically performed on the data, which were obtained in multiple sessions over time. Genotype and treatment were used as independent variables. Food intake or activity parameters were used as dependent variables. If significant effects of genotype or treatment, or a genotype and treatment interaction, over time or not, were found, the independent variables were split for a two- (genotype and treatment) or one-way ANOVA (genotype or treatment) analysis. Following a significant one-way ANOVA, we used the Scheffé F-test for multiple comparisons, with a probability of 0.01 and 0.05 defined as a significant difference. In order to simplify the presentation in the Results section, only the results from the separate ANOVAs conducted on each time period are described.

RESULTS

Similar Baseline Food Intake and Body Weight of 5-HT_{1B} Receptor-Null and Wild-Type Mice Born from Couples of Heterozygous Animals

Two earlier studies have reported that male 5-HT_{1B} receptor-null mice are overweight and consume a higher amount of food than wild-type mice (Brunner *et al*, 1999; Bouwknecht *et al*, 2001). However, the validity of the results was a matter of debate because wild-type and null mice were obtained from independent lines (Bouwknecht *et al*, 2001). Therefore, we used homozygote offspring from heterozygote breeding pairs (Animal section) in order to avoid indirect effects of parental care behavioral responses, as discussed by Bouwknecht *et al* (2001). No difference in either baseline food intake or body weight was detected between wild-type and 5-HT_{1B} receptor-null mice for the habituation period or following a 24 h period of food deprivation (data not shown), as reported previously (Lucas *et al*, 1998).

Kinetics of Feeding Responses after MDMA Treatment in Starved Wild-Type Mice was Biphasic (Hypophagia Followed by Hyperphagia), and Contrasted to a Monophasic Hypophagia Response to 5-HT_{1B} Receptor Agonists

MDMA produced a profound reduction in food intake for 1 h in starved wild-type mice, as compared with NaCl-treated wild-type mice (−77%, Figure 1a). However, after this initial reduction in food intake, we observed that the amount of food consumed over the next 2 h (between 60 and 180 min) was greater in MDMA-treated mice than in NaCl-injected wild-type mice (+47%, Figure 1b). Therefore, the drive to eat induced by starvation was suppressed during the first hour by MDMA. Compared to saline-treated starved mice, MDMA-treated starved mice first displayed a period of hypophagia for 1 h and then a second period of hyperphagia for the next 2 h.

As with MDMA, the 5-HT_{1B} receptor agonist CGS12066A (2 mg/kg, but not 0.5 or 1 mg/kg) reduced food consumption during the first hour following treatment (−65%, Figure 1a). However, in contrast to MDMA, CGS12066A-induced hypophagia was prolonged over 3 h (Figure 1b). Similar results were obtained using the 5-$HT_{1A/1B}$ receptor agonist RU24969 (5 mg/kg, Figure 1a and b), as reported previously (Lucas *et al*, 1998). Thus, 5-HT_{1B} receptor agonists produced a more sustained hypophagia than does MDMA (Figure 1b).

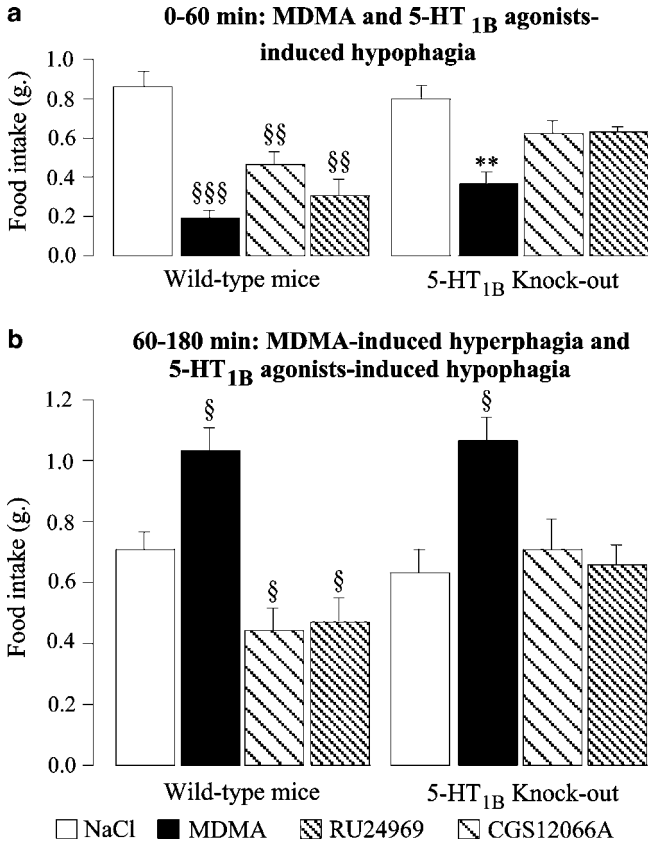

Figure 1 Biphasic feeding responses to MDMA in starved wild-type and 5-HT_{1B} receptor-null mice contrasted to a sustained hypophagia of 5-HT_{1B} receptor agonists. Data are means ± SEM total food intake for groups of wild-type (+/+) and 5-HT_{1B} receptor-null (−/−) mice treated with NaCl (n = 22–17), MDMA (10 mg/kg, n = 11–9), 5-$HT_{1A/1B}$ receptor agonist RU24969 (5 mg/kg, n = 9–7), or 5-HT_{1B} receptor agonist CGS12066A (2 mg/kg, n = 11–7). Food intake was significantly different between mice of both genotypes ($F_{1,85} = 6.2$, $p < 0.05$) and changed after the treatment used for 1 h ($F_{3,85} = 23.4$, $p < 0.0001$) and 60–180 min periods ($F_{3,85} = 13.5$, $p < 0.0001$). Treatments that differ significantly from saline in wild-type mice are marked ($^{§§§}p < 0.0001$, $^{§§}p < 0.01$, $^{§}p < 0.05$) and, in 5-HT_{1B} receptor-null animals, are noted ($^{**}p < 0.01$).

Biphasic Feeding Responses to MDMA were Maintained in 5-HT$_{1B}$ Receptor-Null Mice

Since both MDMA and 5-HT$_{1B}$ receptor agonists reduced food intake in starved mice, we thought that 5-HT$_{1B}$ receptors could be involved in MDMA-delayed eating. However, we did not verify this hypothesis since, in 5-HT$_{1B}$ receptor-null mice, MDMA induced a biphasic effect on feeding behavior similar to the one observed in wild-type mice: a hypophagic effect over 1 h (−58%, Figure 1a) followed by a hyperphagic effect (Figure 1b). As expected, note that, no significant effect of CGS12066A (2 mg/kg) or RU24969 (5 mg/kg) injected alone was observed in 5-HT$_{1B}$ receptor-null mice (Figure 1a and b).

Biphasic Feeding Responses to MDMA were Maintained when 5-HT$_{1B}$ Receptors were Blocked by the Antagonist GR127935

To circumvent any compensatory mechanisms, which may occur in mice lacking 5-HT$_{1B}$ receptors from birth, we tested whether an acute injection of the specific 5-HT$_{1B/1D}$ receptor antagonist, GR127935 (10 mg/kg), combined with MDMA may disrupt the effects of MDMA on feeding. Results indicate that in starved wild-type mice, MDMA plus GR127935 did not modify the biphasic change in food intake provoked by MDMA (Figure 2a and b). In addition, no significant effect on food intake was detected in mice treated with 3 (not shown) or 10 mg/kg of GR127935, as compared to saline-injected wild-type animals (Figure 2a and b).

5-HT$_{2C}$ Receptor Antagonist RS102221 Highly Reduced MDMA-Induced Hypophagia, but not Its Hyperphagic-Delayed Response

5-HT$_{2C}$ receptor-null mice exhibit an increase in food intake (Tecott *et al*, 1995), unrelated to peripheral change (Nonogaki *et al*, 1998). Pharmacological studies have reported that the 5-HT$_{2C}$ receptor antagonist SB242084 reduced the anorectic effects of both *m*-chlorophenylpiperazine, a mixed 5-HT$_{2A,2B,2C}$ receptor agonist (Kennett *et al*, 1997), and fenfluramine (Vickers *et al*, 2001). However, SB242084 alone did not induce hyperphagia, nor did it mimic other behavioral parameters of 5-HT$_{2C}$ receptor-null mice (Kennett *et al*, 1997). Therefore, we chose to use another selective 5-HT$_{2C}$ receptor antagonist, RS102221, because its chronic administration mimics the feeding phenotype of 5-HT$_{2C}$-null mice (Bonhaus *et al*, 1997).

In this new series of experiments, the treatments induced significant changes in food intake over time (1 h: $F_{3,36} = 12.5$, $p < 0.0001$; 60–180 min: $F_{3,36} = 8.3$, $p < 0.001$). Again, we observed a biphasic change in food intake following MDMA injection in starved wild-type animals (Figure 3a and b). RS102221 (2 mg/kg) alone did not change the feeding response for the first period (0–60 min, Figure 3a). Interestingly, if RS102221 (2 mg/kg) was coadministered with MDMA, the hypophagic effect of MDMA observed during this period was greatly reduced compared to the one observed after injection of MDMA alone (Figure 3a). In contrast, for the second period (60–180 min), the hyperphagia detected after MDMA was not reduced after coinjection of RS102221 and MDMA (Figure 3b). Note that RS102221 injected alone induced hyperphagia during this period (Figure 3b).

MDMA did not Induce Hyperlocomotion when Coinjected with RS102221

There is an obvious correlation between horizontal locomotion and feeding responses. To our knowledge, the possible involvement of 5-HT$_{2C}$ receptors in MDMA-induced

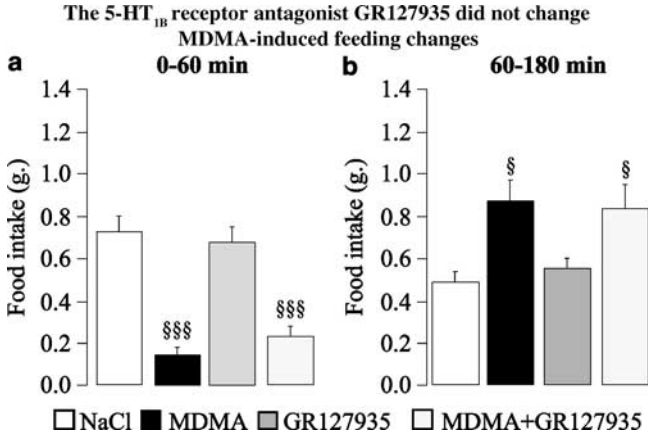

Figure 2 Biphasic feeding responses to MDMA was maintained even when the drug is coadministered with the 5-HT$_{1B/1D}$ receptor antagonist GR127935. Data are means ± SEM total food intake measured 1 h (a) and between 1 and 3 h (b) in wild-type mice treated with NaCl ($n = 10$), MDMA (10 mg/kg, $n = 9$), 5-HT$_{1B/1D}$ receptor antagonist GR127935 (10 mg/kg, $n = 8$), or MDMA/GR127935 ($n = 10$) in starved wild-type mice. Significant differences between any treatment and NaCl effect are noted ($^{§§§}p < 0.0001$, $^{§}p < 0.05$).

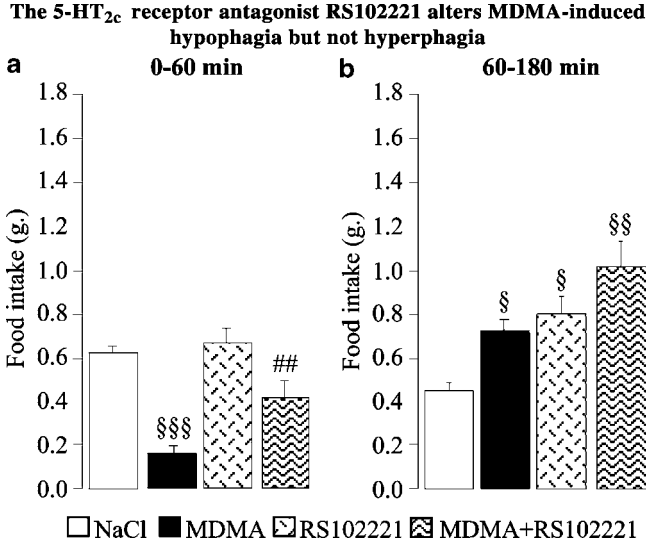

Figure 3 5-HT$_{2C}$ receptor antagonist RS102221 coadministered with MDMA-suppressed MDMA-induced hypophagia, but not hyperphagia. Data are means ± SEM total food intake in starved wild-type mice treated with NaCl ($n = 9$), MDMA (10 mg/kg, $n = 10$), 5-HT$_{2C}$ receptor antagonist RS102221 (2 mg/kg, $n = 12$), or MDMA/RS102221 (2 mg/kg, $n = 13$). Treatments that differ significantly from saline are marked ($^{§§}p < 0.01$, $^{§}p < 0.05$), and those differing from MDMA are noted ($^{##}p < 0.01$).

hyperactivity has never been tested in mice. Our results indicate that MDMA significantly increased the traveled path length, as compared to saline-injected animals (Figure 4a). In contrast, a combined treatment of MDMA plus RS102221 failed to induce any change in horizontal activity (Figure 4a). Similarly, the *total* traveled path length was markedly increased after the administration of MDMA, as compared to control mice (+592%, Figure 4b). This MDMA-induced activity was highly reduced by the coinjection of RS102221 (+226%, $p<0.01$) (Figure 4a and b). Note that RS102221 alone had no effect on locomotor activity (Figure 4a and b).

The effects of MDMA alone on horizontal activity were significantly greater than for mice treated with MDMA plus RS102221 (MDMA *vs* MDMA+RS102221: +226%, $p<0.01$), or RS102221 (MDMA *vs* RS102221: +435%,

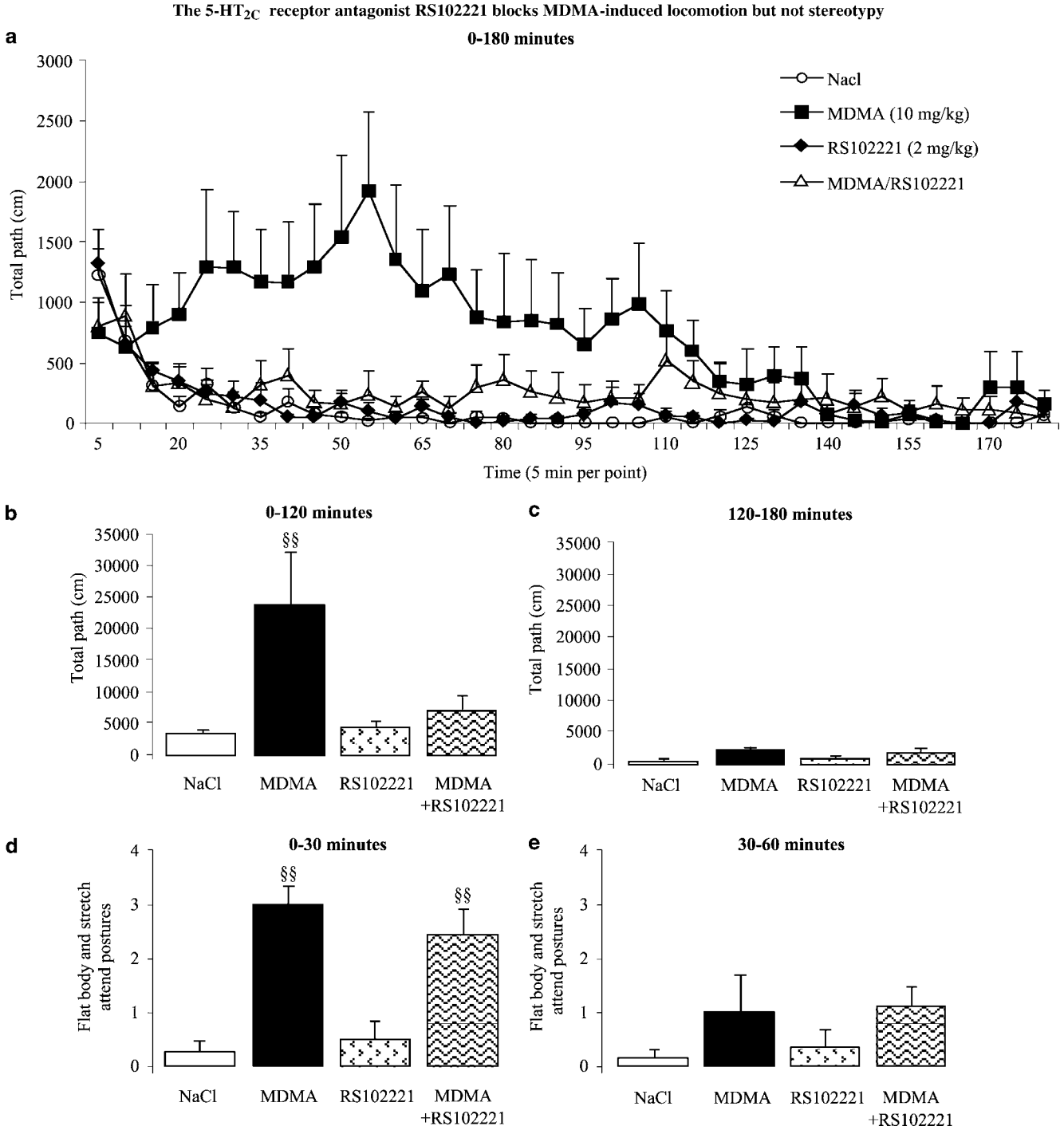

Figure 4 A combined MDMA treatment with the 5-HT$_{2C}$ receptor antagonist RS102221 induced no change in the open field. Näive wild-type animals were placed in identical open fields and their horizontal activity (a–c) and stereotyped behaviors (flat body and stretch–attend postures) (d, e) were, respectively, recorded for 3 h (5 min per point) and for 1 h, for one single day. Significant differences between treatment and NaCl are marked ($^{\S\S}p<0.01$).

$p<0.01$) alone (Figure 4b). After 120 min, there was no significant effect of treatment ($F_{3,26}=0.45$, $p=0.72$, Figure 4c), time ($F_{11,286}=1.27$, $p=0.24$, Figure 4c) or treatment × time interaction ($F_{33,286}=1.01$, $p=0.45$, Figure 4c). In summary, MDMA stimulated locomotion in mice when injected alone, but not when coadministered with RS102221.

Stereotyped behaviors could also compete with both horizontal locomotion and feeding changes after MDMA. The absence of a reduction in the hypophagic response to MDMA when 5-HT_{2C} receptors were inactivated may be explained by the fact that mice exhibited less stereotyped behaviors after cotreatment with MDMA plus RS102221. To investigate such a possibility, we assessed the degree of certain stereotyped behaviors. Wild-type mice treated with MDMA, RS102221, or MDMA plus RS102221 exhibited no significant difference in the number of head weaving, grooming, or licking behaviors, as compared to control animals (not shown). In contrast, both MDMA and MDMA plus RS102221 treatments induced marked increases in the number of flat body and stretch–attend postures observed, as compared to control mice, but only for the initial 30 min following drug injection (Figure 4d). No significant difference was detected after use of any treatment over the next 30 min (Figure 4e). Therefore, the recorded stereotyped behaviors may not compete with feeding or motor responses.

DISCUSSION

There are many studies reporting solid evidence that MDMA administration increases 5-HT steady-state content in several brain structures of rats and mice (McKenna and Peroutka, 1990; Colado *et al*, 2004). Consequently, scientists focused on the effects of the drug on serotonergic system functions, even though MDMA has varying potencies in increasing the synaptic levels of other biogenic amines, such as DA (McKenna and Peroutka, 1990; Colado *et al*, 2004). One dose of 10 mg/kg MDMA (i.p., 3 h), identical to that used under our experimental conditions, was found to increase 5-HT turnover in mouse brain (Steele *et al*, 1989). Few studies indicate that MDMA exerts an effect on food consumption in humans (anorectic or bulimic effects) (Shulgin, 1986; Rochester and Kirchner, 1999; Schifano, 2000), as would be expected from its 'substrate-type 5-HT releaser' activity. Furthermore, only one study reports its hypophagic effect in rats (Frith *et al*, 1987). Curiously, no attempt has been made to investigate the effect of MDMA on feeding behavior in mice and the possible implication of 5-HT receptors. Our results indicate that MDMA induced an initial hypophagia in spite of starvation, suggesting that the drug induced a pharmacological effect sufficient to disrupt the physiological drive in mice to eat. However, this hypophagic effect was transient (1 h) since over the following period (60–180 min) MDMA-treated mice exhibited hyperphagia compared to saline-treated mice. Therefore, the kinetics of the feeding response to MDMA was biphasic. We first thought that 5-HT_{1B} receptors could be implicated in the effects of MDMA in starved mice. However, several points argue against this hypothesis. Firstly, we found that 5-HT_{1B} receptor agonists (CGS12066A and RU24969) effectively reduced food consumption, but they did so differently to MDMA. Their effects were monophasic with a hypophagic response prolonged over the 180 min period of investigation. Secondly, there was no difference observed in the biphasic feeding response to MDMA on feeding behavior in starved 5-HT_{1B} receptor-null mice *vs* wild-type animals. Thirdly, the 5-$HT_{1B/1D}$ receptor antagonist GR127935 did not modify the biphasic effect of MDMA on feeding behavior in starved mice. This result is consistent with that obtained by Vickers *et al* (2001), which showed that GR127935 as well as the 5-$HT_{1A/1B}$ receptor antagonist cyanopindolol did not reduce fenfluramine-induced anorexia in starved rats. However, 5-HT_{1B} receptor-null mice are insensitive to fenfluramine-induced anorexia. These apparently contradictory results may be explained either by a developmental problem or, as suggested, by a reduction of 5-HT_{2C} receptor function in 5-HT_{1B} receptor-null mice (Clifton *et al*, 2003). Compensatory effects of other 5-HT receptors may occur in 5-HT_{1B} receptor-null mice. For example, a compensatory elevation in 5-HT_4 receptor function is likely because the 5-HT_4 receptor antagonist RS39604 reduced MDMA-induced hypophagia (Compan *et al*, 2004). It remains to be determined why the 5-HT_{1B} receptor-null mice are sensitive to MDMA-induced anorexia but not to fenfluramine-induced anorexia. Fenfluramine and MDMA have already been reported to induce different behavioral changes. MDMA administration provoked an increase in locomotion (Rempel *et al*, 1993) and temperature (Malberg and Seiden, 1998), whereas fenfluramine causes hypolocomotion (Bankson and Cunningham, 2001) and hypothermia (Cryan *et al*, 2000).

The implication of 5-HT_{2C} receptors in MDMA-induced hypophagia is clear. The present results indicate that MDMA-induced hypophagia was greatly reduced if MDMA was coadministered with the 5-HT_{2C} receptor antagonist RS102221. Injection of RS102221 also blocked the motor responses to MDMA, indicating that the dose used (2 mg/kg) is effective (see Bonhaus *et al*, 1997 for discussion). Inactivation of 5-HT_{2C} receptors also has been reported to mediate reduced food intake following 5-HT releasers, such as fluoxetine (Lightowler *et al*, 1996), *d*-fenfluramine (Neill and Cooper, 1989; Vickers *et al*, 2001), and norfenfluramine (Vickers *et al*, 2001). Thus, MDMA acts via inducing an increase in 5-HT levels in the synaptic cleft followed by the activation of 5-HT_{2C} receptors. A direct action of MDMA on 5-HT_{2C} receptors cannot be excluded since MDMA does bind to 5-HT_2 receptors with high affinity (0.6–6 μM) (Lyon *et al*, 1986; Battaglia and De Souza, 1989).

MDMA is a drug of abuse that elicits a dramatic increase in locomotion (Geyer and Callaway, 1994), suggesting the possibility that MDMA-induced hypophagia could be due to hyperlocomotion. However, this seems unlikely at least for one reason: MDMA (10 mg/kg)-induced hyperlocomotion was reduced after treatment with GR127935 and in 5-HT_{1B} receptor-null mice (Scearce-Levie *et al*, 1999; Compan *et al*, 2003), whereas the hypophagic effects of MDMA were not. Likewise, several studies have excluded the possibility that nonspecific behavioral changes, such as hyperlocomotion and even sedation, may account for the hypophagic responses to 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B}, or 5-HT_{2C} receptor agonists (Kitchener and Dourish, 1994; Lee and Simansky,

1997; Hewitt *et al*, 2002; Clifton *et al*, 2000, 2003). The fact that 5-HT_{2C} receptors may contribute to MDMA-induced hyperlocomotion has been questioned in one study using rats (Bankson and Cunningham, 2002). This investigation reported that SB206553, a 5-HT_{2C} receptor antagonist, enhanced the motor effects of MDMA in the rat (Bankson and Cunningham, 2002). Such a discrepancy may likely be due to dose- or species-specific differences since an acute administration of 3.3 mg/kg MDMA did not stimulate locomotion in mice (Scearce-Levie *et al*, 1999), but did in rats (Bankson and Cunningham, 2002). This difference may depend on the fact that a 10 mg/kg dose of MDMA induces a marked increase in the DA release in rats, while only a modest rise is obtained at the same dose in mice (see Yamamoto *et al*, 1995; Colado *et al*, 2004). Moreover, the use of different antagonists may also lead to varied results. It has been shown recently that SB206553 is not a neutral antagonist, but an inverse agonist of 5-HT_{2C} receptors. This compound elevated the extracellular DA levels in the nucleus accumbens of rats by a significantly greater magnitude than did the 5-HT_{2C} receptor antagonist SB242084 (De Deurwaerdère *et al*, 2004). Consequently, the influence of serotonergic systems, in part by 5-HT_{2C} receptors, appears to be dependent on the degree of elevation of DA concentration, as extensively debated by Bankson and Cunningham, in studies using rats (2001).

Our results suggest that 5-HT_{2C} receptor transmission may underlie the initial hypophagic effect of MDMA, but that is likely not responsible for the following period of hyperphagia. Indeed, the delayed hyperphagic effect of MDMA (period 60–180 min) was not modified by the coinjection of RS102221 and MDMA. Nevertheless, this is possible that hyperphagia could be mediated via one or several other neuronal systems such as peptidergic and/or dopaminergic systems.

These findings present the first evidence that a pharmacological inactivation of 5-HT_{2C} receptors using RS102221 overcomes both MDMA-induced feeding and motor disorders in mice.

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