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Administration of SCH 23390 into the Medial Prefrontal Cortex Blocks the Expression of MDMA-Induced Behavioral Sensitization in Rats: An Effect Mediated by 5-HT_{2C} Receptor Stimulation and not by D_1 Receptor Blockade

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Akin to what has been reported for cocaine, systemic administration of the dopamine D1 receptor antagonist, SCH 23390 ((*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride), blocks the expression but not the induction of 3,4-methylenedioxymethamphetamine (MDMA)-induced behavioral sensitization. Since the medial prefrontal cortex (mPFC) appears to regulate the expression of sensitization to cocaine, this study examined whether microinjection of SCH 23390 into the mPFC would alter the expression of MDMA sensitization. Saline or MDMA was administered for 5 consecutive days. After 12 days of withdrawal, rats received a bilateral intra-mPFC microinjection of SCH 23390 or saline followed by an intraperitoneal (i.p.) challenge dose of MDMA. While SCH 23390 enhanced locomotion in MDMA-naïve rats, it completely suppressed the expression of sensitization in MDMA-pretreated animals. Since, SCH 23390 has a fairly good affinity for 5-HT_{2C} receptors, we went further to study the role of mPFC D1 and 5-HT_{2C} receptors in this, apparently, paradoxical effect shown by SCH 23390. Thus, the microinjection of both SKF 81297 (*R*-(+)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide) and MK 212 (6-chloro-2-(1-piperazinyl)pyrazine hydrochloride), a D1 and 5-HT_{2C} receptor agonist, respectively, blocked MDMA sensitization. By contrast, the 5-HT_{2C} receptor antagonist, RS 102221 (8-[5-(2,4-dimethoxy-5-(4-trifluoromethylphenylsulfonamido)phenyl-5-oxopentyl]-1,3,8-triazaspiro[4,5]decane-2,4-dione hydrochloride), had no effect in MDMA-naïve or MDMA-sensitized animals, but reversed the effects of SCH 23390 in MDMA-pretreated rats. These results demonstrate that suppression of MDMA-induced sensitization by SCH 23390 is mediated by 5-HT_{2C} receptor stimulation in the mPFC and not by the blockade of mPFC D1 receptors. Furthermore, these data indicate that stimulation of 5-HT_{2C} receptors by SCH 23390 is not a minor issue and should be considered when interpreting future data.
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INTRODUCTION

Intermittent administration of psychostimulants in laboratory animals induces an enduring and progressive increase in the behavioral effects of subsequent drug injections that persists after long periods of withdrawal (Pierce and Kalivas, 1997; Wolf, 1998; Vanderschuren and Kalivas, 2000). This phenomenon, termed behavioral sensitization, has been considered for many years a useful animal model for the development of psychosis (Kalivas and Stewart, 1991; Pierce and Kalivas, 1997). However, recent studies have also emphasized that the neuronal plasticity underlying sensitization results in the enhancement of the incentive motivational effects of psychostimulants, which contributes to drug craving (Robinson and Berridge, 1993; Di Chiara, 1995; Robinson and Berridge, 2000).

3,4-Methylenedioxymethamphetamine (MDMA, 'ecstasy') is an amphetamine derivative that has become a very popular drug despite its potential neurotoxic effects and psychiatric complications reported in recreational MDMA users (Green *et al*, 2003). MDMA has the ability of lowering the threshold for rewarding intracranial self-stimulation (Hubner *et al*, 1988), produces conditioned place preference (Bilsky *et al*, 1998), and it is self-administered by rats and primates (Beardsley *et al*, 1986; Schenk *et al*, 2003). MDMA not only shares these rewarding properties with other

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psychostimulants such as amphetamine and cocaine but also elicits long-term behavioral and neurochemical sensitization in rats (Spanos and Yamamoto, 1989; Kalivas *et al*, 1998; McCreary *et al*, 1999).

Evidence has accumulated suggesting a crucial role for mesocorticolimbic dopaminergic system that projects from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC), in behavioral sensitization to psychostimulants. Neuroadaptations in the VTA play an important role in the development of such phenomenon, while the NAc and mPFC have a key role in the expression of behavioral sensitization (Pierce and Kalivas, 1997; White and Kalivas, 1998; Wolf, 1998; Vanderschuren and Kalivas, 2000). Furthermore, many researchers have postulated that the dopamine D1 receptor is important for psychostimulant-induced behavioral sensitization (Stewart and Vezina, 1989; Kalivas and Stewart, 1991; Henry and White, 1991; Bjijou *et al*, 1996; Vezina, 1996). However, the role of D1 receptors appears to vary depending on the psychostimulant and the process being studied, that is, development *vs* expression. More specifically, D1 receptor antagonist, SCH 23390 ((*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride), blocks both the development and expression of amphetamine- and methamphetamine-induced behavioral sensitization (Vezina and Stewart, 1989; Hamamura *et al*, 1991; Vezina, 1996; Karper *et al*, 2002). In contrast, D1 receptor antagonism blocks the expression but not the development of cocaine sensitization (Mattingly *et al*, 1994; White *et al*, 1998).

We have recently shown that SCH 23390, akin to cocaine sensitization, blocks the expression but not the induction of MDMA sensitization (Ramos *et al*, 2004). Owing to this similarity and because the mPFC regulates the expression of cocaine sensitization (Pierce *et al*, 1998; Prasad *et al*, 1999; Sorg *et al*, 2001), in the present work we tested whether D1 receptors located in the mPFC could contribute to the results found in our previous study after systemic SCH 23390 (Ramos *et al*, 2004). Our results show that SCH 23390 administered into the mPFC increases the locomotor activity induced by and acute injection of MDMA (5 mg/kg intraperitoneally (i.p.)), but blocks the sensitized response to a challenge dose of MDMA. SCH 23390 has been extensively used as a dopamine D1 receptor antagonist (Bourne, 2001); however, it also binds with high affinity to 5-HT_{2C} receptors (Briggs *et al*, 1991; Millan *et al*, 2001). For this reason, we went further to evaluate whether 5-HT_{2C} receptor stimulation by SCH 23390 could account for the blockade of the expression of MDMA-induced behavioral sensitization.

MATERIALS AND METHODS

Drugs

The sources of the drugs used were as follows: racemic (+/−)-MDMA-HCl was a gift from the 'Servicio de Restricción de Estupefacientes' (Madrid, Spain). The dose of (+/−)-MDMA is reported as concentration of the salt and was prepared by dissolving in physiological saline. MK 212 (6-chloro-2-(1-piperazinyl)pyrazine hydrochloride), RS 102221 (8-[5-(2,4-dimethoxy-5-(4-trifluoromethylphenylsulfonamido)phenyl-5-oxopentyl]-1,3,8-triazaspiro[4,5]-decane-2,4-dione hydrochloride), and SCH 23390 were purchased from Tocris (Biogen Científica SL, Madrid, Spain). SKF 81297 (*R*-(+)-6-Chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide) was obtained from Sigma-Aldrich (Madrid, Spain). All solutions injected centrally were adjusted to pH 7.2, except the solution of RS 102221, which was adjusted to pH 6–7; control vehicle at pH 6–7 did not alter MDMA-stimulated locomotor activity when compared to other saline-treated groups.

Animals

Experiments were carried out in male Wistar rats (Harlan, Barcelona), weighing 260–290 g at the beginning of drug treatment. Animals were housed four per cage in constant conditions of humidity and temperature ($22 \pm 1^{\circ}C$) with a 12-h/12-h light–dark cycle (lights on at 0700). Food and water were available *ad libitum*. Animals were handled on the 2 days preceding the beginning of drug treatment and on 2 days preceding drug challenges. The treatment schedule for all experimental groups is shown in Table 1. All the procedures followed in the present work were in compliance with the European Community Council Directive of 24 November 1986 (86/609/EEC) and were approved by the Ethical Committee of the University of Navarra.

Locomotor Sensitization Procedure

Horizontal locomotor activity was measured in an open field, which consisted of nine square arenas ($43 \times 51 \times 45\ cm^3$) made of black wood using a video tracking system (Ethovision 3.0, Noldus Information Technology BV, Wageningen, The Netherlands), in a softly illuminated

Table 1 Behavioral Sensitization Protocol and Treatment Regimens

Groups (induction/day 18)	*n*	Day1	Day 2 (morning/evening)	Days 3–6 (morning and evening)	Day 11	Day 17	Day 18 (intra-mPFC/i.p.)
Saline/saline	7–9	Saline	Saline/saline	Saline	Surgery	Saline	Saline/MDMA-5
MDMA/saline	8–11	Saline	MDMA-5/MDMA-10	MDMA-15	Surgery	Saline	Saline/MDMA-5
Saline/drug	7–12	Saline	Saline/saline	Saline	Surgery	Saline	Drug/MDMA-5
MDMA/drug	8–9	Saline	MDMA-5/MDMA-10	MDMA-15	Surgery	Saline	Drug/MDMA-5

Rats received morning injections between 0900 and 1300, while evening injections were given between 1900 and 2200. The drugs used on day 18 were as follows: D_1 receptor antagonist, SCH 23390 (0.1, 0.025, or 0.01 μg/side); D_1 receptor agonist, SKF 81297 (0.1 μg/side); 5-HT_{2C} receptor agonist, MK 212 (0.005 μg/side); and 5-HT_{2C} receptor antagonist, RS 102221 (0.15 μg/side).

experimental room. The tracking system was set to determine the position of the animal 5 times/s. All morning experiments were conducted between 0900 and 1300. Evening injections were given between 1900 and 2200. Distance traveled by rats was only measured in the morning sessions of days 2 and 18 (see below).

During the whole course of the experiments, we used the MDMA administration protocol previously described by Ramos *et al* (2004). All rats were placed into the arenas 24 h prior to beginning repeated MDMA (15 mg/kg i.p.) or saline administrations. On this first day, rats were injected with saline (1.0 ml/kg i.p.) and were placed into the testing arenas for a 120 min period to let animals adapt to the new environment. The next day (day 2), the subjects were divided into MDMA and saline treatment groups. Subjects were administered with either saline or MDMA (5 mg/kg i.p.) and were placed into the arenas. Measurement of locomotor activity began after the first 15 min. Data analysis did not begin immediately after drug administration to avoid confounds due to the injection procedure. After the habituation period, motor activity was monitored for 105 min. In the evening, rats were injected with saline or MDMA (10 mg/kg i.p.) and were placed into the arenas for 60 min. Over the next 4 days (days 3–6), rats received two injections of either saline or MDMA (15 mg/kg i.p.). The first injection was given in the morning (between 0900 and 1300), while the second one was given between 1900 and 2200. Thereafter, rats were put into the arenas for 60 min. On day 11, rats went under surgery for cannulae implantation into the mPFC (see below). At 11 days after the last dose of MDMA or saline (day 17), rats were injected with saline and were placed into the testing arenas for a 120 min. The next day (day 18), all the rats received an intra-mPFC infusion of either saline or the drugs under study (SCH 23390, SKF 81297, MK 212, or RS 102221) followed by a challenge dose of MDMA (5 mg/kg i.p.). The animals were placed into the testing arenas for a 120 min period, where locomotor activity was quantified after the first 15 min habituation period. In all cases, animals were challenged only once.

Surgical Procedures and Intracranial Injections

At 1 week before the challenge dose of MDMA, rats were anesthetized with a combination of ketamine (70 mg/kg i.p.) and xylazine (7 mg/kg i.p.) and placed in a Kopf stereotaxic frame, with the incisor bar set at 3.3 mm below the interaural line. The skull was exposed and two holes were drilled to allow implantation of two single 26-G guide cannulae (C315G Plastics1, Roanoke, VA, USA) into the mPFC according to the Atlas of Paxinos and Watson (1997) (all coordinates given relative to bregma): medial PFC, $+3.2$ mm anteroposterior (AP), ± 0.7 mm mediolateral (ML), and -2.2 mm dorsoventral (DV). Guide cannulae were lowered into place and attached to the skull via two small stainless-steel screws and dental acrylic. Obturators (C315DC, Plastics1) cut to extend 0.5 mm beyond the tip of each cannula were inserted to prevent obstruction by debris. After surgery, the animals were housed individually with free access to food and water. To minimize infection, all animals were injected with the antibiotic enrofloxacin (0.5 mg/kg i.p.; Baytril™) once a day for 5 days.

On the day of the experiment (day 18), saline or the drug under study were microinjected into the mPFC using a stainless-steel 33-G internal cannula (C315I, Plastics1), connected to PE-20 tubing leading to a 10 μl Hamilton syringe. The internal cannulae extended 1 mm below the guide cannulae bilaterally, and a volume of 0.5 μl/side was delivered over a period of 1 min. The internal cannulae were allowed to remain in place for 2 additional minutes following the injection. After 15 min, a challenge dose of MDMA (5 mg/kg i.p.) was administered to the rats and the animals were placed into the arenas for 15 min before measurements of locomotor activity. Coordinates for local microinjections were chosen based on previous findings showing that only the prelimbic subarea, and not the infralimbic or anterior cingulate subareas of the mPFC, is involved in the expression of cocaine-induced sensitization (Pierce *et al*, 1998; Tzschentke and Schmidt, 2000).

Verification of Cannulae Placement

At the completion of experiments, animals were killed by decapitation, and the brains were rapidly removed and were frozen in powder dry ice. Coronal sections (25 μm) were cut at the level of the PFC using a cryostat. The sections were mounted on gelatin-coated slides, stained with thionine, and placed under coverslips to verify probe placements under a light microscope by an individual unaware of the rat's behavioral response.

Statistical Analysis

Horizontal locomotor activity expressed as traveled distance (cm) was calculated in 15-min blocks. Total horizontal activity data (a panels in Figures 1, 3–6) were analyzed with two-way ANOVA with repeated measures over days. Time-course analyses of all behavioral data (b panels in Figures 1, 3–6) were performed with a two-way ANOVA with repeated measures over time. Data from different treatment groups within 1 day were analyzed with one-way ANOVA followed by Tukey's *post hoc* test and data from each group within different days were analyzed with Student's *t*-test paired data.

RESULTS

Local Microinjection of SCH 23390 into the mPFC Prevents the Expression of MDMA Sensitization

As expected, the stimulant effect of a challenge dose of MDMA (5 mg/kg i.p.) on day 18 was significantly enhanced in the MDMA-pretreated animals compared with saline-pretreated rats and with the distance traveled by these same rats on day 2 ($t\ (8) = -5.848$, $P < 0.001$). To examine whether blockade of mPFC D1 receptor stimulation would prevent the expression of MDMA-induced sensitization, SCH 23390 (0.1, 0.025, or 0.01 μg/0.5 μl) was injected bilaterally into the mPFC 15 min before the challenge dose of MDMA on day 18. As it can be seen in Figure 1 (top panel) and Figure 2, all three doses of SCH 23390 increased locomotion in MDMA-naïve rats. By contrast, although the lowest dose of SCH 23390 tested (0.01 μg) caused no remarkable effect, the infusion of the middle dose of SCH

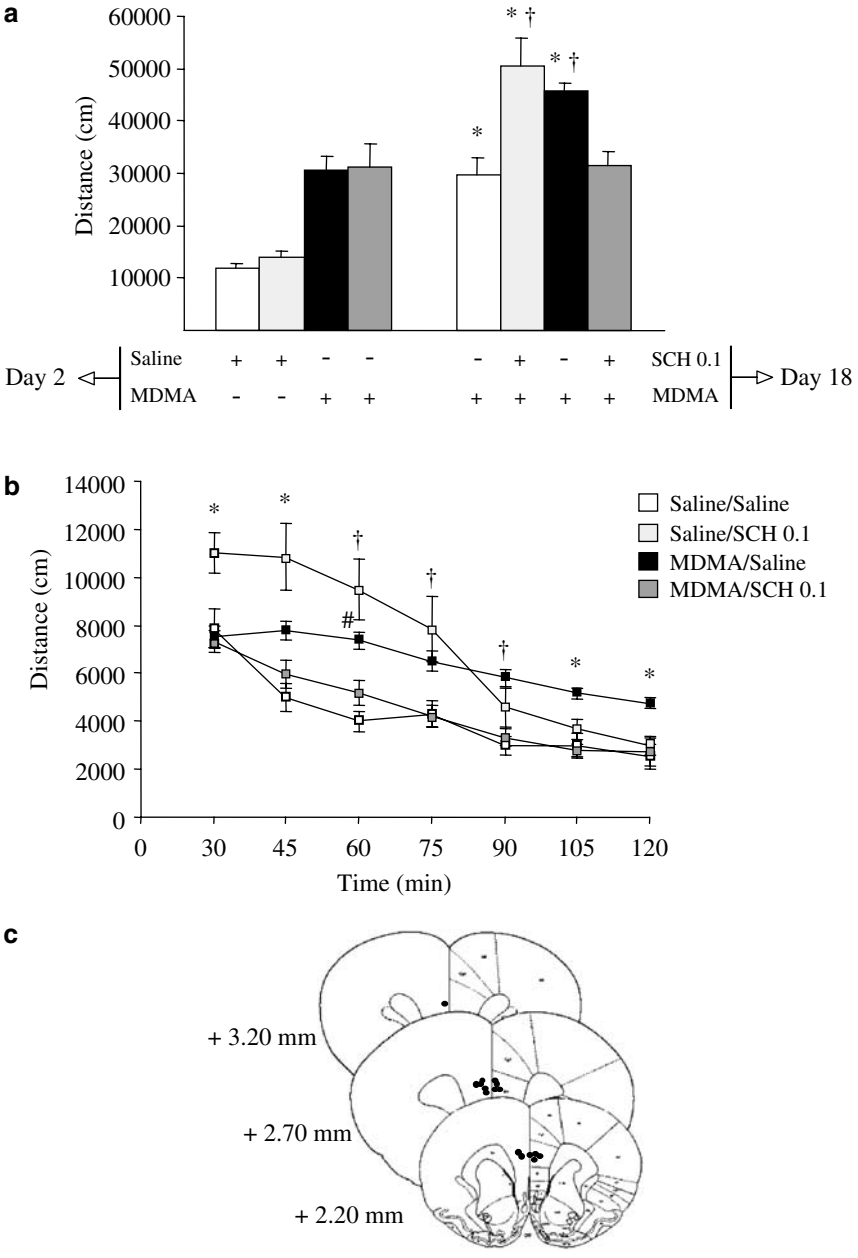

Figure 1 Effect of intra-mPFC infusion of SCH 23390 on the expression of MDMA-induced sensitization. The bar graph in panel a illustrates the mean ± SEM distance traveled (cm) over 105 min, beginning 15 min after the first injection of saline or MDMA (5 mg/kg i.p.) in the morning of day 2 or the challenge dose of MDMA on day 18. The line graph in panel b shows the time course of horizontal activity in 15 min time blocks for105 min after injecting MDMA on day 18. The data were statistically evaluated using a two-way ANOVA with repeated measures over day of injection (panel a) or time (panel b). Panel a: TreatmentF(3,29) = 10.068, $p < 0.001$; dayF(1,29) = 82.725, $p < 0.001$, interactionF(3,29) = 13.762, $p < 0.001$. *$p < 0.05$ compared with day 2 within each treatment group; $^{\dagger}p < 0.05$ compared with the saline-pretreated rats on day 18. Panel b: TreatmentF(3,29) = 8.11, $p < 0.001$; timeF(6,174) = 83.373, $p < 0.001$; interactionF(18,174) = 7.469, $p < 0.001$. *$p < 0.05$ *vs* the rest of the groups; $^{\dagger}p < 0.05$ i.saline/saline and MDMA/SCH 23390; $^{\#}p < 0.05$ *vs* saline/saline. (c) Coronal sections taken from the Atlas of Paxinos and Watson (1997) at the level of the prefrontal cortex. The numbers indicate mm anterior to bregma. Spots correspond to the cannulae placements from the data plotted in panels a and b (group MDMA/SCH 23390).

23390 (0.025 μg) caused a nonsignificant trend towards the prevention of the sensitized response in MDMA-pretreated animals. Such effect reached significance in the group of rats treated with the highest dose of SCH 23390 (0.1 μg) (Figure 1). In this last case, the two-way ANOVA for repeated measures revealed a significant interaction (treatment × time $F(18, 174) = 7.469$, $P < 0.001$). Thus, every single 15 min time block was analyzed using a one-way ANOVA followed by Tukey's test. Statistical differences between treatments at each time block are also shown in panel b of Figure 1.

Microinjection of the Dopamine D1 Receptor Agonist SKF 81297, or the 5-HT_{2C} Receptor Agonist MK 212 into the mPFC Prevent MDMA-Induced Sensitization

In the following set of experiments, we administered the D1 receptor agonist, SKF 81297 (0.1 μg/0.5 μl/side), into the

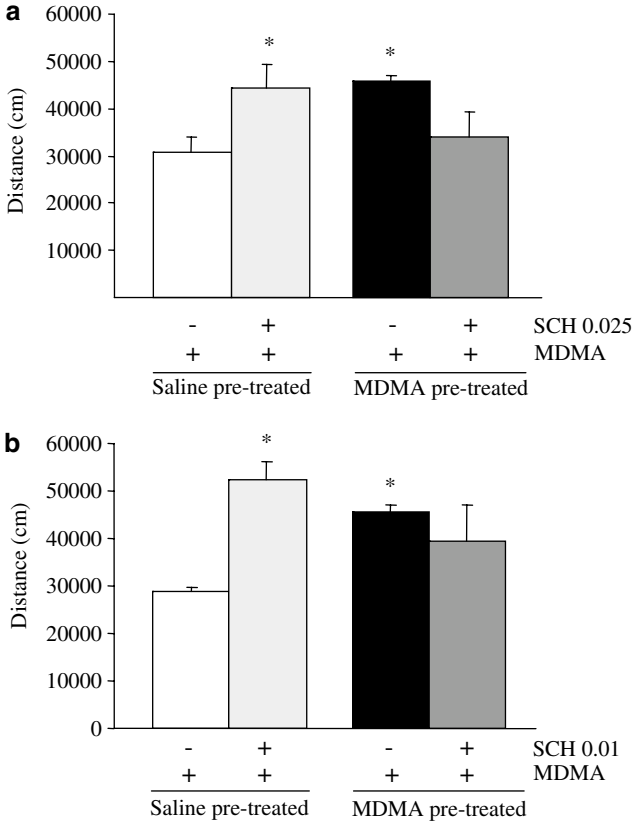

Figure 2 Effect of intra-mPFC infusion of different doses of SCH 23390 on the expression of MDMA-induced sensitization. Rats were treated with either saline or the MDMA sensitization protocol (see also Table 1 for further details). On day 18, an intra-mPFC microinjection of saline or SCH 23390 (0.025 μg/0.5 μl/side, panel a) or (0.01 μg/0.5 μl/side, panel b) was given. After 15 min, rats received either saline or were challenged with a dose of MDMA (5 mg/kg i.p.). In all cases, animals were challenged only once. Data represent mean ± SEM of distance traveled (cm) over 105 min, beginning 15 min after the challenge dose of MDMA on day 18. Panel a: TreatmentF(3,29) = 4.632, $p < 0.01$. Panel b: TreatmentF(3,31) = 5.628, $p < 0.01$. *$p < 0.05$ compared with saline/saline group.

mPFC. Infusion of SKF 81297, akin to SCH 23390, completely blocked the expression of MDMA-induced behavioral sensitization, although it did not alter the activating effects of MDMA in the saline-pretreated group (Figure 3). The dose of SKF 81297 was chosen according to a previous study by Sorg *et al* (2001), showing that this dose of SKF 81297 blocks the expression of cocaine sensitization.

Similarly, the 5-HT_{2C} receptor agonist MK 212 (0.005 μg/0.5 μl/side) administered to MDMA-naïve animals did not affect the stimulant effects of the challenge dose of MDMA, but prevented the sensitized response in MDMA-pretreated animals (Figure 4). In this case, the dose of MK 212 was chosen based upon a previous study showing that MK 212 (0.05 μg/side) does not alter the activating effects of cocaine (Filip and Cunningham, 2003). Under our experimental conditions, however, such a dose did significantly attenuate the distance traveled by an acute dose of MDMA (data not shown). For this reason, we used a 10-fold lower dose of MK 212, which, in any case, resulted in the prevention of MDMA-induced sensitization.

The Suppression of MDMA Sensitization by SCH 23390 is Reversed by Coadministration of the 5-HT_{2C} Receptor Antagonist, RS 102221

In a final set of experiments, the 5-HT_{2C} receptor antagonist, RS 102221, was microinjected alone or in combination with SCH 23390, 15 min before the challenge dose of MDMA on day 18. In the first case, RS 102221 (0.15 μg/0.5 μl/side) by itself did not modify the activating effects of MDMA in saline- or MDMA-pretreated animals (Figure 5). On the contrary, when RS 102221 was coinfused with SCH 23390, the blockade of MDMA sensitization by 0.1 μg of SCH 23390 was reversed by RS 102221 (Figure 6). However, the combination of both drugs did not modify the acute response to MDMA in drug-naïve rats.

DISCUSSION

The main findings of our study are: (1) SCH 23390 in the mPFC suppresses the expression of behavioral sensitization to repeated MDMA; (2) D1 receptor activation by SKF 81297 in the mPFC also blocks the expression of MDMA sensitization; (3) these same results were achieved after 5-HT_{2C} receptor stimulation by MK 212; and (4) the 5-HT_{2C} receptor antagonist, RS 102221, reverses the prevention of MDMA-induced sensitization by SCH 23390.

We have previously shown that systemic injections of the putative D1 receptor antagonist, SCH 23390, block the expression but not the induction of MDMA-induced behavioral sensitization (Ramos *et al*, 2004). These effects are similar to what has been previously described for cocaine (McCreary and Marsden, 1993; Mattingly *et al*, 1994; Tella, 1994; Martin-Iverson and Reimer, 1994; White *et al*, 1998). Furthermore, akin to what has also been shown for cocaine (Pierce and Kalivas, 1997), the microinjection of SCH 23390 into the core of the NAc prevents the expression of MDMA sensitization (Ramos *et al*, 2004). Owing to these similarities between cocaine and MDMA, and because the expression of cocaine sensitization is regulated by dopamine transmission in the mPFC (Prasad *et al*, 1999; Sorg *et al*, 2001), we went further to examine whether the local application of SCH 23390 in this brain region would also affect the expression of MDMA sensitization.

A large amount of data have implicated the glutamatergic projections arising from the mPFC to the NAc and to the VTA in the development of both amphetamine and cocaine sensitization (eg Wolf *et al*, 1995; Cador *et al*, 1999; Li *et al*, 1999a; see also review by Vanderschuren and Kalivas, 2000). However, while different studies have excluded any role of the mPFC in the expression of amphetamine sensitization (eg Li and Wolf, 1997), results from different laboratories suggest that alterations in glutamate signaling are involved in the expression of cocaine sensitization. Thus, ibotenic acid lesions of the dorsal mPFC (but not the ventral mPFC) block the expression of cocaine sensitization and the sensitized glutamate release in the NAc (Pierce *et al*, 1998; but see Li *et al*, 1999b). Furthermore, intra-mPFC microinjection of amphetamine or the D1 receptor agonist SKF 81297 prevents the expression of cocaine sensitization (Prasad *et al*, 1999; Sorg *et al*, 2001; see also Vanderschuren and Kalivas, 2000 for a review).

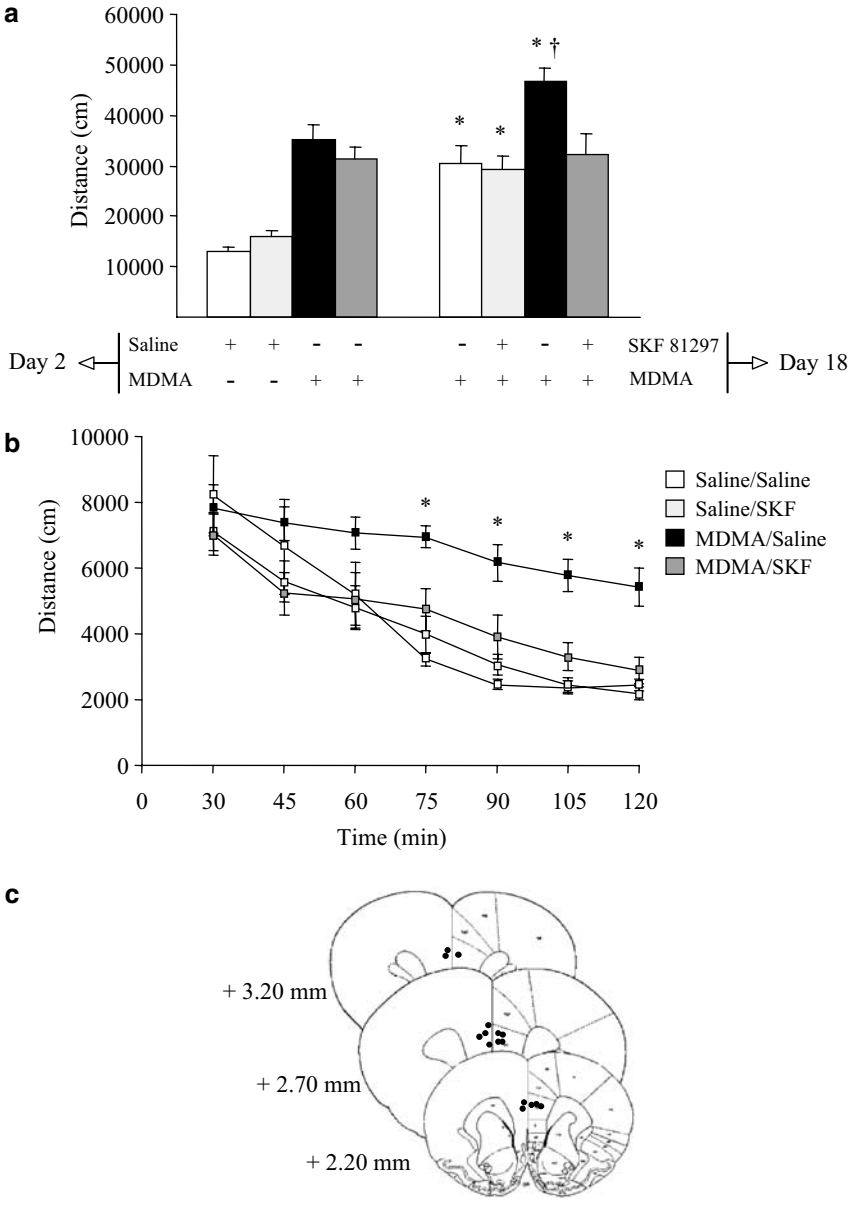

Figure 3 Effect of intra-mPFC infusion of SKF 81297 (0.1 μg/0.5 μl/side) on the expression of MDMA-induced sensitization. The bar graph in panel a illustrates the mean ± SEM distance traveled (cm) over 105 min, beginning 15 min after the first injection of saline or MDMA (5 mg/kg i.p.) in the morning of day 2 or the challenge dose of MDMA on day 18. The line graph in panel b shows the time course of horizontal activity in 15 min time blocks for 105 min after injecting MDMA on day 18. The data were statistically evaluated using a two-way ANOVA with repeated measures over day of injection or time (panel b). Panel a: Treatment$F(3,42) = 15.2$, $p < 0.001$; day$F(1,42) = 36.3$, $p < 0.001$, interaction$F(3,42) = 3.5$, $p < 0.05$. *$p < 0.05$ compared with day 2 within each treatment group; †$p < 0.05$ compared with the saline-pretreated rats on day 18. Panel b: Treatment$F(3,42) = 3.778$, $p < 0.05$; time$F(6,252) = 54.677$, $p < 0.001$; interaction$F(18,204) = 2.041$, $p < 0.05$. *$p < 0.05$ *vs* the rest of the groups. (c) Coronal sections taken from the Atlas of Paxinos and Watson (1997) at the level of the prefrontal cortex. The numbers indicate mm anterior to bregma. Spots correspond to the cannulae placements from the data plotted in panels a and b (group MDMA/SKF 81297).

In this study, we now report that blockade of mPFC D1 receptors with SCH 23390 increases locomotion in MDMA-naïve rats, which is in agreement with the contention that the relationship between mPFC dopamine and locomotion occurs directly by dopamine's inhibitory action on excitatory amino-acid neurons in the mPFC. This hypothesis is congruent with the ability of cortical DA transmission for inhibiting spontaneous (Bubser and Schmidt, 1990), novelty-induced (Radcliffe and Erwin, 1996) and psychostimulant-induced (Vezina *et al*, 1991; Banks and Gratton, 1995; Beyer and Steketee, 2002; Steketee, 2003) locomotor activity. Our results also show that, akin to cocaine (Prasad *et al*, 1999; Sorg *et al*, 2001), the D1 receptor agonist SKF 81297 blocks the expression of MDMA-induced behavioral sensitization, providing further support for the putative inhibitory role of dopaminergic innervation of the mPFC on cortical excitatory efferent projections that innervate subcortical areas.

Accordingly, as it does in drug-naïve animals, D1 receptor blockade by SCH 23390 in the mPFC would have been

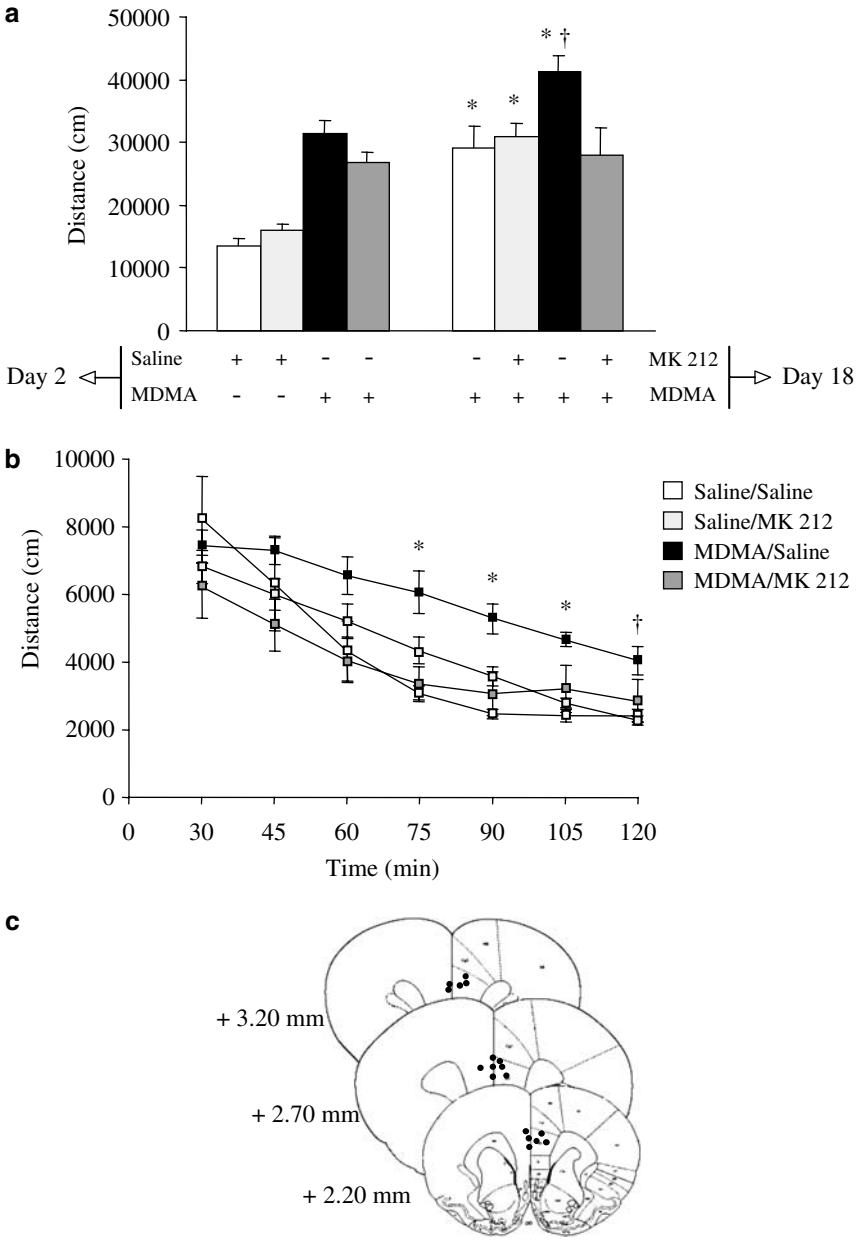

Figure 4 Effect of intra-mPFC infusion of MK-212 (0.005 μg/0.5 μl/side) on the expression of MDMA-induced sensitization. The bar graph in panel a illustrates the mean ± SEM distance traveled (cm) over 105 min, beginning 15 min after the first injection of saline or MDMA (5 mg/kg i.p.) in the morning of day 2 or the challenge dose of MDMA on day 18. The line graph in panel b shows the time course of horizontal activity in 15 min time blocks for 105 min after injecting MDMA on day 18. The data were statistically evaluated using a two-way ANOVA with repeated measures over day of injection or time (panel b). Panel a: TreatmentF(3,34) = 11.469, $p < 0.001$; dayF(1,34) = 53.825, $p < 0.001$, interactionF(3,34) = 5.555, $p < 0.05$. *$p < 0.05$ compared with day 2 within each treatment group; $^{\dagger}p < 0.05$ compared with the saline-pretreated rats on day 18. Panel b: TreatmentF(3,34) = 4.592, $p < 0.05$; timeF(6,204) = 47.867, $p < 0.001$; interactionF(18,252) = 2.184, $p < 0.05$. *$p < 0.05$ *vs* the rest of the groups; $^{\dagger}p < 0.05$ *vs* saline/saline and saline/MK-212. (c) Coronal sections taken from the Atlas of Paxinos and Watson (1997) at the level of the prefrontal cortex. The numbers indicate mm anterior to bregma. Spots correspond to the cannulae placements from the data plotted in panels a and b (group MDMA/MK-212).

expected to increase locomotion in MDMA-sensitized rats and not the opposite. Then, how does SCH 23390 block MDMA sensitization? The answer probably relies on its pharmacological properties. Thus, and although taken into account in very few studies, SCH 23390 also stimulates 5-HT_{2C} receptors, exhibiting a fairly good affinity for these receptors ($K_i \approx 0.3$ nM for D1 receptors *vs* $K_i \approx 6.3$ nM for 5-HT_{2C} receptors; Briggs *et al*, 1991; Bourne, 2001; Millan *et al*, 2001). Iontophoretic application of 5-HT ligands suppresses spontaneous firing of PFC neurons in a 5-HT_{2C} receptor-dependent manner (Bergqvist *et al*, 1999), suggesting that the 5-HT_{2C} receptor limits the excitability of cortical pyramidal neurons (Carr *et al*, 2002). Therefore, it could be thought that SCH 23390 is capable of blocking MDMA sensitization by stimulating mPFC 5-HT_{2C} receptors. The support for this hypothesis comes from our data

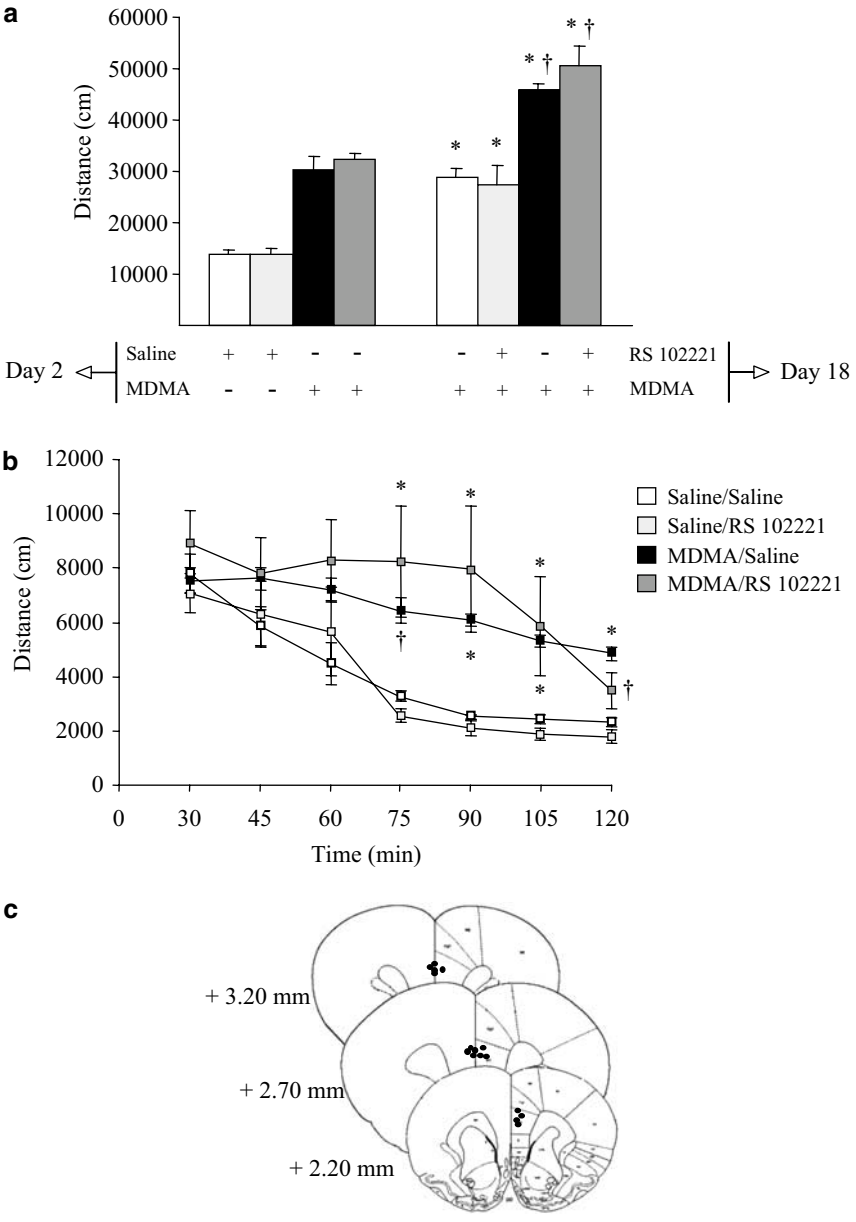

Figure 5 Effect of intra-mPFC infusion of RS 102221 (0.15 μg/0.5 μl/side) on the expression of MDMA-induced sensitization. The bar graph in panel a illustrates the mean ± SEM distance traveled (cm) over 105 min, beginning 15 min after the first injection of saline or MDMA (5 mg/kg i.p.) in the morning of day 2 or the challenge dose of MDMA on day 18. The line graph in panel b shows the time course of horizontal activity in 15 min time blocks for105 min after injecting MDMA on day 18. The data were statistically evaluated using a two-way ANOVA with repeated measures over day of injection or time (panel b). Panel a: TreatmentF(3,32) = 12.695, $p < 0.001$; dayF(1,32) = 49.668, $p < 0.001$, interactionF(3,32) = 0.194, NS. *$p < 0.05$ compared with day 2 within each treatment group; †$p < 0.05$ compared with the saline-pretreated rats on day 18. Panel b: TreatmentF(3,32) = 5.357, $p < 0.05$; timeF(6,192) = 29.429, $p < 0.001$; interactionF(18,192) = 2.519, $p < 0.001$. *$p < 0.05$ *vs* saline/saline and saline/RS 102221; †$p < 0.05$ *vs* saline/RS 102221. (c) Coronal sections taken from the Atlas of Paxinos and Watson (1997) at the level of the prefrontal cortex. The numbers indicate mm anterior to bregma. Spots correspond to the cannulae placements from the data plotted in panels a and b (group MDMA/RS 102221).

showing that the 5-HT_{2C} receptor agonist MK 212 completely prevents the expression of sensitization in MDMA-pretreated animals.

Previous studies by Pan and Wang (1991a, b) showed that the inhibition of pyramidal cells in the prefrontal cortex produced by MDMA is mediated mainly through the serotonergic system. Moreover, it has been shown that damage produced by the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) significantly increases the inhibitory effects of serotonin on frontal cortex pyramidal cells, while the response of these cells to the iontophoresis of dopamine is attenuated (Ashby *et al*, 1994). Therefore, although speculative, lesions of the serotonergic terminals induced by repeated MDMA injections (Aguirre *et al*, 1995) could render these cells more sensitive to the inhibitory effects of 5-HT_{2C} receptor stimulation by SCH 23390. Further support for this contention comes from the demonstration that classical denervation supersensitivity of 5-HT_{2C} develops

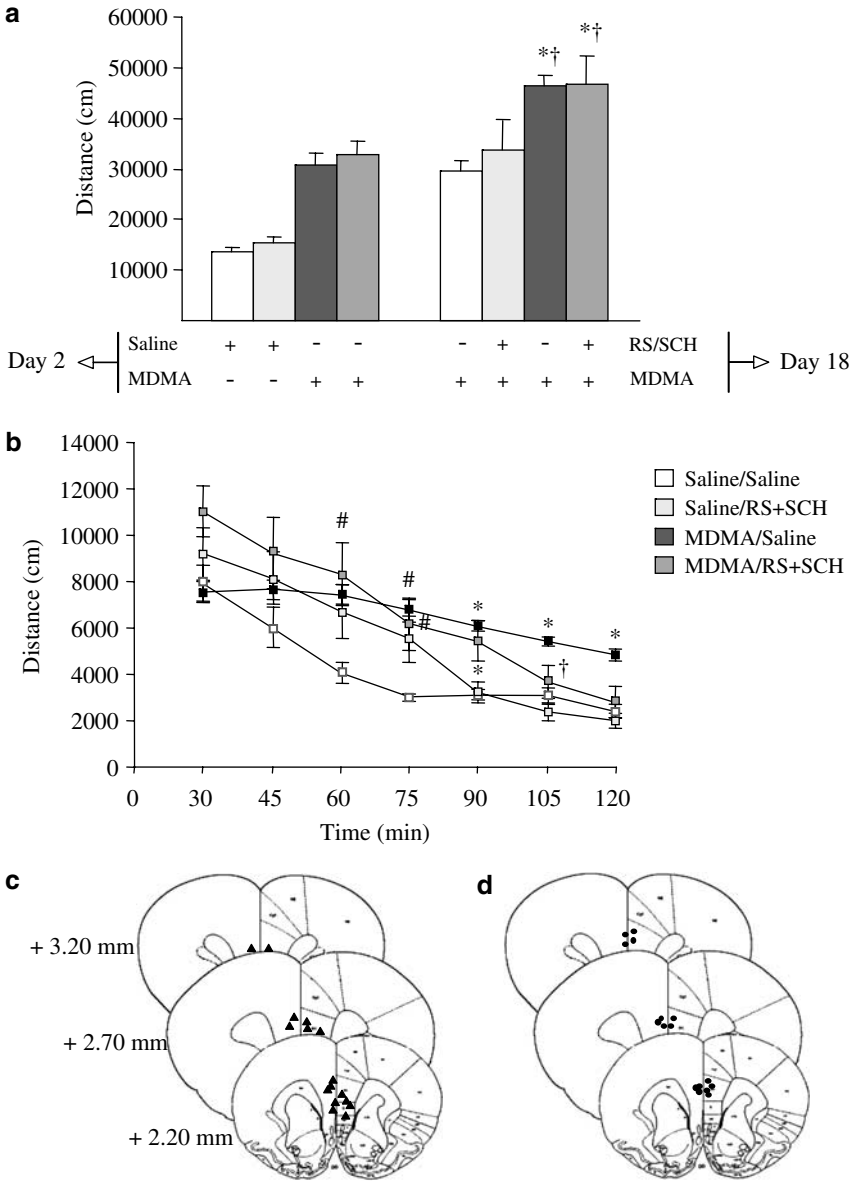

Figure 6 Effect of intra-mPFC infusion of SCH 23390 + RS 102221 (0.1 + 0.15 μg/0.5 μl/side) on the expression of MDMA-induced sensitization. The bar graph in panel a illustrates the mean ± SEM distance traveled (cm) over 105 min, beginning 15 min after the first injection of saline or MDMA (5 mg/kg i.p.) in the morning of day 2 or the challenge dose of MDMA on day 18. The line graph in panel b shows the time course of horizontal activity in 15 min time blocks for105 min after injecting MDMA on day 18. The data were statistically evaluated using a two-way ANOVA with repeated measures over day of injection or time (panel b). Panel a: TreatmentF(3,34) = 12.442, $p < 0.001$; dayF(1,34) = 57.898, $p < 0.001$, interactionF(3,34) = 0.138, NS. $^{*}p < 0.05$ compared with day 2 within each treatment group; $^{\dagger}p < 0.05$ compared with the saline-pretreated rats on day 18. Panel b: TreatmentF(3,34) = 3.725, $p < 0.05$; timeF(6,204) = 69.813, $p < 0.001$; interactionF(18,204) = 3.854, $p < 0.001$. $^{*}p < 0.05$ *vs* the rest of the groups; $^{\dagger}p < 0.05$ *vs* saline/RS + SCH; $^{\#}p < 0.05$ *vs* saline/saline. Panels c and d: Coronal sections taken from the Atlas of Paxinos and Watson (1997) at the level of the prefrontal cortex. The numbers indicate mm anterior to bregma. Triangles (saline-pretreated/RS 102221 + SCH 23390 group) and spots (MDMA-pretreated/RS 102221 + SCH 23390 group) correspond to the cannulae placements from the data plotted in panels a and b.

following depletion of 5-HT with 5,7-DHT. For example, 5-HT_{2C} receptor ligand binding (Conn *et al*, 1987), the 5-HT_{2C}-like immunoreactivity (Sharma *et al*, 1997), and the behavioral consequences of 5-HT_{2C} receptor stimulation (Lucki *et al*, 1989) are increased following 5,7-DHT pretreatment. It is also note worthy that a similar MDMA treatment as the one used in this study produces a long-term increase of 5-HT_{2C} receptor mRNA expression in the hippocampus (Yau *et al*, 1994).

On the other hand, there is a large body of evidence showing that MDMA-induced 5-HT release contributes to MDMA's effect on dopamine release (Koch and Galloway, 1997). Thus, pharmacological inhibition of MDMA-induced 5-HT release attenuates MDMA-induced striatal DA release (Gudelsky and Nash, 1996; Koch and Galloway, 1997). Similarly, the nonselective 5-$HT_{2A/2C}$ receptor antagonist ritanserin attenuates MDMA-induced increases in DA release in the nigrostriatal pathway (Yamamoto *et al*,

1995), while 5-HT_{2A} receptor activation is necessary for MDMA-induced DA release (Schmidt *et al*, 1992). We do not know of any previous work showing that a neurotoxic MDMA treatment similar to the one used in our experiments decreases dopamine release in the mPFC after a challenge dose of MDMA. However, in a recent study by Matuszewich *et al* (2002), it was shown that the depletion of 5-HT after repeated injections of MDMA leads to an attenuation of extracellular dopamine concentrations in the prefrontal cortex in response to a behavioral challenge (ie immobilization stress). These later authors suggested that the removal of the normal 5-HT-mediated inhibition of GABAergic tone on dopamine cell bodies of the VTA (Bankson and Yamamoto, 2004) may explain the inhibition of stress-induced cortical dopamine release after neurotoxic doses of MDMA. According to the above-mentioned studies, it could be speculated that 5-HT depletion caused by the MDMA sensitization protocol would decrease the forebrain dopamine release in response to a challenge dose of MDMA. This might explain the opposite results found after SCH 23390 in drug-naïve rats *vs* MDMA-pretreated animals on day 18. Thus, in saline-pretreated rats, a systemic injection of MDMA would increase 5-HT and dopamine release in the mPFC. In this case, the main effect of SCH 23390 should be the blockade of D1 receptors and not 5-HT_{2C} receptor stimulation. This, in turn, would increase locomotor activity (see Figures 1 and 2). By contrast, in MDMA-pretreated rats, a blunted dopamine release would occur in the mPFC in response to a challenge dose of MDMA. In this case, the main effect of SCH 23390 is more likely dependent on its ability to stimulate 5-HT_{2C} receptors after a challenge dose of MDMA on day 18. This, in turn, would result in the blockade of MDMA-induced sensitization (Figure 1). This is in accordance with the data showing that the lowest dose of SCH 23390 used (0.01 μg/side) was relatively ineffective at inhibiting MDMA-sensitized behavior, yet had a pronounced effect on the acute behavioral activity of MDMA. Actually, SCH 23390 increased locomotor activity without any apparent dose–response effect. By contrast, there exists a dose–response effect of SCH 23390 in the blockade of MDMA sensitization. Owing to its affinity profile, this may reflect a more selective effect of SCH 23390 as a D1 receptor antagonist at lower doses, while higher doses of SCH 23390 are necessary to block MDMA sensitization through 5-HT_{2C} receptor stimulation.

Our data also show that the 5-HT_{2C} receptor antagonist RS 102221 reverses the blockade of MDMA sensitization caused by SCH 23390, further supporting our hypothesis that SCH 23390 prevents the expression of MDMA-induced sensitization by activating mPFC 5-HT_{2C} receptors and not by blocking D1 receptors located in this brain region. It should be noted, however, that the RS/SCH combination did not increase locomotion in MDMA-naïve rats as it would have been expected. We do not have a clear explanation for this fact. It is known that RS 102221 or SCH 23390 injected into the NAc block the hyperactivity of cocaine and/or MDMA (McMahon *et al*, 2001; Filip and Cunningham, 2002; Ramos *et al*, 2004). It is possible then that the RS/SCH combination diffused to the Nac as some of the animals in this group exhibited cannulae placements in a very close proximity to the ventral portion of the prefrontal cortex (ie the infralimbic mPFC, see Figure 6C).

Finally, it is important to note that DA in the mPFC has been shown to be a modulatory neurotransmitter (see review by Seamans and Yang, 2004). Therefore, depending on the contribution and interaction of DA with other cortical systems, including glutamate (Ramos *et al*, 2005) and GABA (Simantov and Peng, 2004), different responses may occur. These complex interactions between multiple neurotransmitter systems and/or brain regions warrant also consideration when interpreting the results of this and other studies.

In summary, our findings show that D1 or 5-HT_{2C} receptor stimulation in the mPFC is sufficient to prevent the expression of MDMA sensitization. In a similar manner, they also indicate that the blockade of MDMA sensitization by SCH 23390 is mediated by 5-HT_{2C} receptor stimulation excluding any role for mPFC D1 receptor blockade. Therefore, we believe that under some circumstances, the stimulation of 5-HT_{2C} receptors by SCH 23390 is not a minor issue and should be considered when interpreting future data. In any case, due to the popularity of this drug of abuse among young people, more studies examining the long-term consequences after repeated MDMA administration are needed.

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