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ORIGINAL INVESTIGATION

MDMA modifies active avoidance learning and recall in mice

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Abstract
Rationale Several studies have suggested the existence of cognitive deficits after repeated or high doses of 3,4-methylenedioxymethamphetamine (MDMA) in humans and experimental animals. However, the extent of the impairments observed in learning or memory tasks remains unclear.
Objective The objective of this study was to evaluate the effects of different dosing regimens of MDMA on the ability of mice to learn and recall an active avoidance task.
Materials and methods Animals were treated with MDMA (0, 1, 3, 10 and 30 mg/kg) under four different experimental conditions, and active avoidance acquisition and recall were evaluated. In experiments 1 and 2, MDMA was administered 1 h before different active avoidance training sessions. In experiments 3 and 4, mice received a repeated treatment with MDMA before or after active avoidance training, respectively. Changes in presynaptic striatal dopamine transporter (DAT) binding sites were evaluated at two different time points in animals receiving a high dose of MDMA (30 mg/kg) or saline twice a day over 4 days.
Results MDMA administered before the active avoidance sessions interfered with the acquisition and the execution of a previously learned task. A repeated treatment with high doses of MDMA administered before training reduced acquisition of active avoidance in mice, while pre-treatment with both high and low doses of MDMA impaired recall of this task. A reduction in DAT binding was observed 4 days but not 23 days after the last MDMA administration.
Conclusions Acute MDMA modifies the acquisition and execution of active avoidance in mice, while repeated pre-treatment with MDMA impairs acquisition and recall of this task.

Keywords Ecstasy · Memory · DAT binding · Shuttle-box · Conditioning

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Introduction

The recreational drug 3,4-methylenedioxymethamphetamine (MDMA) is widely consumed around the world. Although abundant literature has pointed to acute or long-term functional consequences of MDMA consumption in cognitive processing, the underlying mechanisms and the extent of the deficits produced by MDMA administration remains unclear. Numerous studies have associated heavy or chronic ecstasy use to persistent cognitive deficits in humans (Morgan 1999; Bhattachary and Powell 2001; Dafters 2006; Quednow et al. 2006; Zakzanis and Campbell 2006; Kalechstein et al. 2007). Experiments in animals have shown learning and memory alterations in rats (Sprague et al. 2003; Able et al. 2006; Skelton et al. 2006), monkeys (Taffe et al.

2001; Frederick and Paule 1997), and mice (Glennon et al. 1987; Rosecrans and Glennon 1987) using a variety of behavioural paradigms. However, pinpointing the underlying mechanism related to these deficits has been difficult, primarily due to the varying profiles of MDMA effects in different species (see Easton and Marsden 2006 for review). Currently, there is strong evidence suggesting that high doses of MDMA produce changes in the serotonergic system in rats and monkeys without affecting the dopaminergic system (Ricaurte et al. 1988; Mayerhofer et al. 2001; Green et al. 2003; Colado et al. 2004). While in mice, MDMA predominantly produces changes in the dopaminergic system as shown by reduction in striatal dopamine transporter (DAT) binding sites (Green et al. 2003; Kindlundh-Högberg et al. 2007) or decreases in dopamine (DA) metabolites (Green et al. 2003; Colado et al. 2004).

Research directed towards associating the learning and memory impairments after repeated or high doses of MDMA with neurochemical changes in rats and monkeys has been conflicting. Thus, cognitive, social and affective changes have been observed in the absence of severe serotonergic damage in rats (Fone et al. 2002; Piper and Meyer 2004). Furthermore, MDMA exposure during early development in rats produces dose-related impairments in sequential and spatial learning and memory with small changes in brain region-specific dopamine (DA), 5-HT and norepinephrine, which do not correlate with the learning deficits (Broening et al. 2001). On the other hand, other studies show no differences in lever-press responding (Byrne et al. 2000) or in T-maze performance (Ricaurte et al. 1993) after neurotoxic regimens of MDMA in rats. Similarly, in monkeys, no significant effects were observed in neuropsychological tests of memory, reinforcer efficacy and bimanual motor coordination (Taffe et al. 2001) or performance in an operant test battery (Frederick and Paule 1997), after MDMA at doses producing long-lasting changes in brain neurotransmitter systems. Thus, MDMA-induced cognitive deficits in monkeys and rats appear to be independent of neurochemical changes in the serotonergic system. In mice, however, very few studies have evaluated the effects of MDMA on learning and memory processes (Glennon et al. 1987; Rosecrans and Glennon 1987), and no studies have been conducted using the active avoidance paradigm, essentially measuring associative learning and memory. In addition, it is unclear whether such changes are related to the prominent effect of MDMA on DA terminals.

Therefore, the aim of this study was to evaluate the effects of different dosing regimens of MDMA on the ability of mice to learn and recall an active avoidance task. Studies evaluating changes in dopamine transporter (DAT) binding sites were also performed to assess the effects of such regimens on DA terminals.

Materials and methods

Animals

Experiments were performed in CD1 male mice, weighing 25 to 30 g at their arrival at the laboratory. Mice were initially housed five per cage in a temperature (21±1°C) and humidity (65±10%) controlled room with a 12-/12-h light/dark cycle (lights on from 0800 to 2000 hours) with ad libitum food and water. Experiments took place during the light phase. Behavioural tests and animal care were conducted in accordance with the standard ethical guidelines (National Institutes of Health 1995; European Communities Directive 86/609 EEC) and approved by the local ethical committee (CEEA-IMAS-UPF). All experiments were carried out under blind conditions.

Drugs

MDMA hydrochloride [(±) 3,4-methylenedioxymethamphetamine] was obtained from Lipomed, A.G. (Arlesheim, Switzerland), and dissolved in 0.9% physiological saline.

Active avoidance procedure

Mice were trained to avoid an aversive unconditioned stimulus (US), an electric shock (0.2 mA) continuously applied to the grid of the floor, associated with the presentation of a light (10 W) serving as a conditioned stimulus (CS) in a two-way shuttle box apparatus (Panlab, Barcelona). The apparatus consists of a box with two compartments (20×10 cm) connected by a 3×3-cm door. The CS was switched on in the compartment in which the mouse was placed preceding in 5 s the onset of the US and overlapped it for 25 s. Using this procedure, the light was presented in the compartment for 30 s (5 s alone and 25 s together with the US). At the end of the 30-s period, both CS and US were automatically turned off. A conditioned response was recorded when the animal avoided the US by changing from the compartment where the animal received the CS into the opposite compartment within the 5 s after the onset of the CS. If animals failed to avoid the shock, they could escape it by crossing during the US (25 s). Between each trial session, there was an intertrial interval of 30 s. The ratio of conditioned with respect to the total number of changes of compartment was also determined. Mice were treated with different regimens of MDMA (0, 1, 3 and 10 mg/kg) and 30 mg/kg (twice a day) during 4 days, and at the appropriate time, they were placed in the shuttle box for 10 min before the start of each session to allow them to explore the box. After this habituation period, mice were subjected daily to 100-trial active avoidance sessions.

Four experiments were completed to study learning and memory processes in the shuttle boxes in mice exposed to different regimens of MDMA. In experiments 1 and 2, acquisition and performance in the shuttle boxes were evaluated when the animals were under the acute effects of MDMA. In experiments 3 and 4, the animals were trained in the shuttle boxes before or after a repeated treatment with MDMA but avoiding the acute effects of the drug.

Experiments 1 and 2: Acute effects of MDMA on active avoidance learning and recall

The acute effects of MDMA on active avoidance learning were evaluated in two experimental conditions. In experiment 1, different groups of animals were treated once daily for 4 consecutive days with MDMA (1, 3 and 10 mg/kg, i.p.) or saline 1 h before each training session. A high dose of MDMA (30 mg/kg, i.p.) was also administrated to a different group of animals twice a day (4 h apart) during 4 days. On days 5 and 12, active avoidance performance was evaluated after saline administration.

In experiment 2, animals received saline for 4 consecutive days 1 h before each training session. On days 5 to 8, MDMA (0, 1, 3 and 10 mg/kg, i.p.) was administered once daily 1 h before each testing session. A high dose of MDMA (30 mg/kg, i.p.) was also administered in experiment 2 twice a day during 4 days. On day 9, all animals received saline and were again tested in this procedure.

Experiments 3 and 4: Effects of MDMA administration before and after active avoidance training on learning and recall

Two experimental conditions were used to evaluate the consequences of a repeated MDMA treatment outside the training period. In experiment 3, mice received a repeated treatment with MDMA (0, 1, 3, 10 and 30 mg/kg, i.p.) twice a day during 4 days before training. The effects of this pretreatment were evaluated during a 10-day learning period and three recall tests in the shuttle boxes (see Fig. 3a).

In experiment 4, mice previously trained in the active avoidance task during ten sessions were treated twice a day during 4 days with MDMA (0, 1, 3, 10 and 30 mg/kg, i.p.). After these treatments, the animals were tested again on alternate days during three recall sessions in the shuttle boxes (see Fig. 3b).

Autoradiography of [^{3}H]-mazindol binding

To assess changes in presynaptic DAT binding sites induced by MDMA, 14 mice receiving MDMA (30 mg/kg) twice a day for 4 days and ten mice treated with saline were sacrificed 4 days after the last injection. An additional group of mice from experiment 3 treated with MDMA (0 and 30 mg/kg) twice a day for 4 days were killed 24 h after the last recall test (23 days after the last treatment). The [^{3}H] mazindol autoradiographic studies were carried out according to the protocol used by Ryan et al. (2001) with slight modifications, as previously described (Robledo et al. 2004). Briefly, animals were decapitated and their brains were quickly removed and frozen by immersion in 2-methyl-butane surrounded by dry ice. All samples were stored at −80°C before use. Coronal sections 20 μm-thick were cut in a cryostat and thaw-mounted on gelatine/chrome-coated slides. Sections were then pre-incubated at 4°C for 15 min in 50 mM Trish buffer (pH 7.9) containing 300 mM NaCl and 5 mM KCL before incubation for 60 min in the same buffer containing 300 nM desipramine (Sigma Chemical, Madrid Spain) and 4 nM [^{3}H] mazindol (New England Nuclear; specific activity 24 Ci/mmol). Desipramine was included to block [^{3}H] mazindol binding to noradrenaline uptake sites. Sections were then washed twice for 3 min in the Tris–HCl pre-incubation buffer and dried under a stream of cold-dry air. To determine the non-specific binding, consecutive sections were incubated in the same buffer for 60 min at 4°C with the addition of 100 μM nomifensine (Sigma Chemical). Autoradiograms were generated by apposing the labelled tissues, together with autoradiographic standards ([^{3}H] micro-scales, Amersham), to tritium-sensitive film (Hyperfilm-[^{3}H], Amersham) for a period of 6 weeks and developed for 5 min at 20°C. Films were analysed and quantified with a computer-assisted video-densitometer (MCID, St. Catherine, Ontario Canada) using the standard curve generated from [^{3}H]-standards. Specific binding measured in the entire striatum was determined by subtracting the non-specific binding image from that of total binding.

Statistical analysis

Differences in the number of conditioned changes and in the ratio of conditioned vs total changes were analysed using a two-way analysis of variance (ANOVA; day×dose), followed by one-way ANOVAs and the Dunnett post-hoc test for comparisons with saline. Biochemical studies were analysed using one-way ANOVA. All the analyses were conducted using the statistical package Statistical Package for the Social Sciences (SPSS) 11.0 for Windows (SPSS, Chicago, IL, USA). Differences were considered significant if the probability of error was less than 5%. Data are represented as mean+SEM.

Results

Experiment 1: Acute effects of MDMA on active avoidance acquisition

The acute exposure to MDMA dose-dependently improved the performance of an active avoidance task in experimentally naïve mice. Thus, mice treated with MDMA showed more conditioned changes than controls from the first training session (Fig. 1a). Repeated measures ANOVA revealed a significant main effect of day of training [$F(3,162)=234.732$, $p<0.000$], a significant effect of the dose [$F(4,54)=18.221$, $p<0.000$] and a significant interaction between these two factors [$F(12,162)=6.973$, $p<0.000$]. Post-hoc comparisons following significant one-way ANOVAs for each day of training showed that the acute administration of MDMA significantly increased the number of conditioned changes in the group treated with 3 and 10 mg/kg, as well as in the group treated with 30 mg/kg twice a day for 4 days with respect to mice treated with saline, while no increases were observed after treatment with MDMA at the dose of 1 mg/kg. In addition, mice treated with MDMA at the dose of 3 mg/kg exhibited a higher ratio of conditioned changes during most of the training sessions (Fig. 1b). Repeated measures ANOVA revealed a significant main effect of day of training [$F(3,165)=129.066$, $p<0.000$], a significant effect of the dose [$F(4,55)=4.126$, $p<0.01$], and a significant interaction between these two factors [$F(12,165)=1.813$, $p<0.05$]. Post-hoc comparisons following significant one-way ANOVAs for each day of training revealed that the acute administration of MDMA significantly increased the ratio of conditioned changes with respect to saline at the doses of 3, 10 and 30 mg/kg. During the test session on day 5, where mice received only saline, the group previously treated with 30 mg/kg of MDMA twice a day for 4 days showed less conditioned changes when compared to the saline group as shown by one-way ANOVA [$F(4,59)=7.878$, $p<0.000$]. No differences between groups were observed during the test on day 12 (Fig. 1a). For the ratio of conditioned changes, no significant differences were observed between groups on the test days 5 and 12 (Fig. 1b).

Experiment 2: Acute effects of MDMA on active avoidance performance after a drug-free acquisition

The acute administration of MDMA after a previous drug-free training period of 4 days differentially modified the number and the ratio of conditioned changes (Fig. 2). For conditioned changes, repeated measures ANOVA revealed a significant main effect of day of training [$F(3,108)=27.599$, $p<0.000$], dose [$F(4,36)=4.601$, $p<0.01$] and a significant interaction between these two factors [$F(12,108)=5.024$, $p<0.000$].

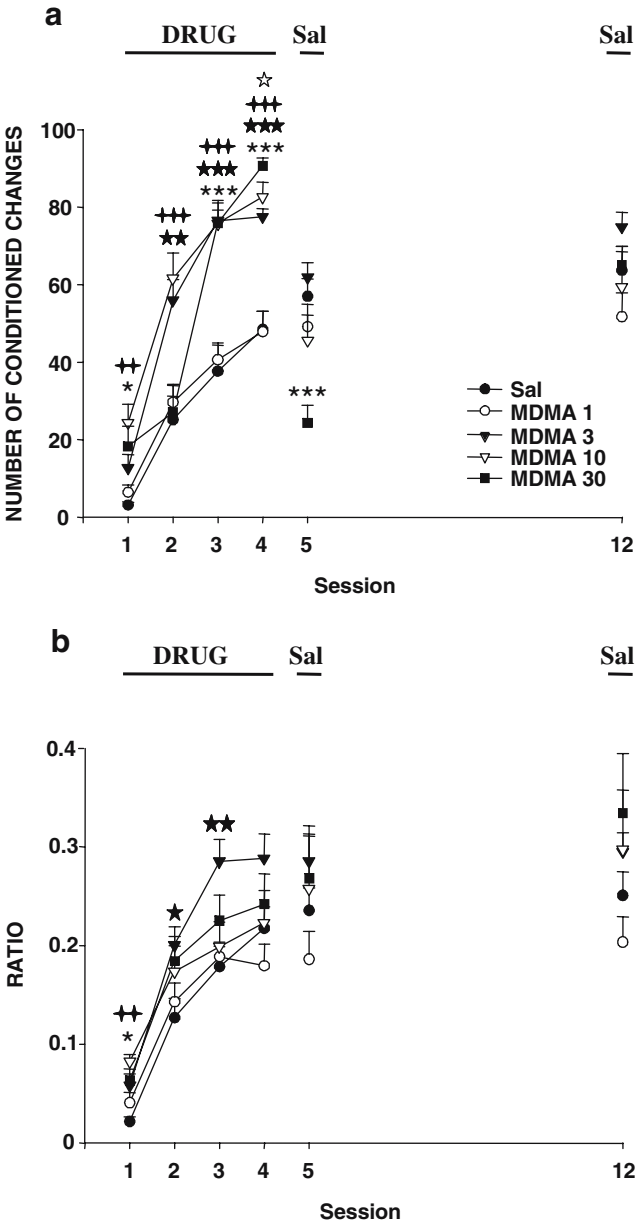

Fig. 1 Acute effects of MDMA during active avoidance acquisition in experimentally naive mice. Average number of conditioned changes (**a**) and ratio of conditioned changes with respect to the total number of changes of compartment (**b**) (+SEM) in the shuttle boxes after the acute injection of saline ($n=12$), MDMA 1 mg/kg ($n=11$), 3 mg/kg ($n=17$), 10 mg/kg ($n=10$) and 30 mg/kg (twice a day for 4 days; $n=10$). Mice were trained during 4 days in 100 trial sessions. On days 5 and 12, all mice received saline and were tested on the task. $p<0.05$ (MDMA 1 mg/kg vs saline); $p<0.001$ (MDMA 3 mg/kg vs saline); $p<0.01$, $p<0.001$ (MDMA 10 mg/kg vs saline); $p<0.05$, $p<0.001$ (MDMA 30 mg/kg vs saline, Dunnett post-hoc test)

Post-hoc comparisons following significant one-way ANOVAs for each day of training revealed that the number of conditioned changes significantly increased in mice pretreated with MDMA at the dose of 10 mg/kg on days 6 ($p<0.05$) and 8 ($p<0.01$) with respect to those treated with saline. However, these effects were no longer significant on

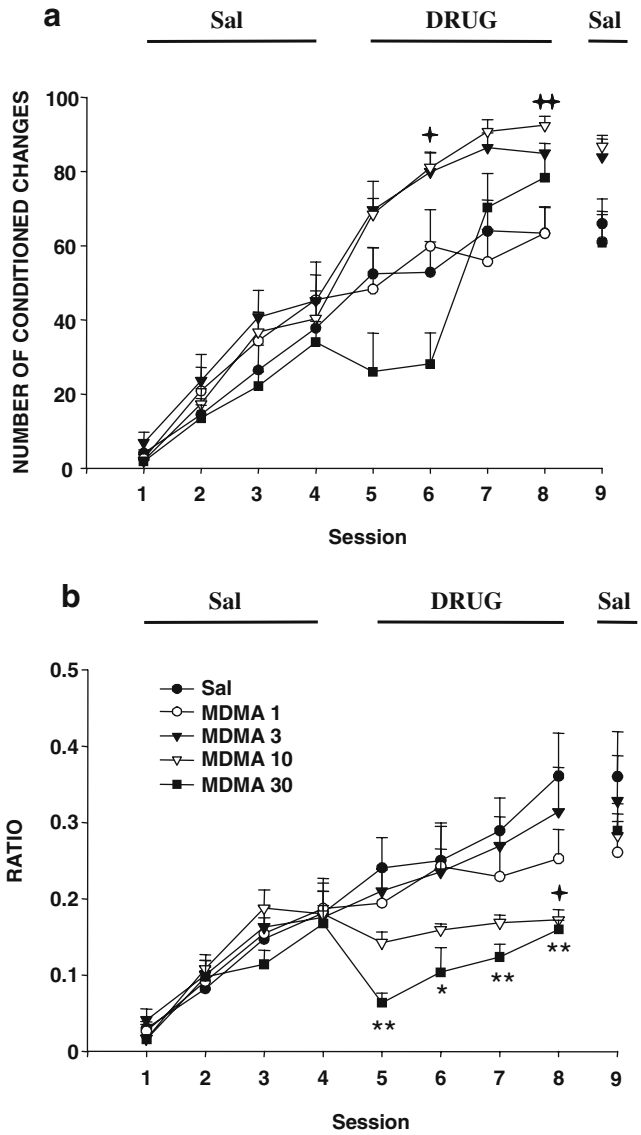

Fig. 2 Acute effects of MDMA on active avoidance performance after a drug-free acquisition. Average number of conditioned changes (**a**) and ratio of conditioned changes (**b**) (+SEM) in the shuttle boxes in 100 trial sessions during 4 days of drug-free training and in four consecutive training sessions after the acute injection of saline (n=11), MDMA 1 mg/kg (n=7), 3 mg/kg (n=7), 10 mg/kg (n=7), and 30 mg/kg (twice a day for 4 days; n=9). On day 9, all mice were tested on the task after saline administration. $p<0.05$, $p<0.01$ (MDMA 10 mg/kg vs saline); $p<0.05$, $p<0.01$ (MDMA 30 mg/kg vs. saline, Dunnett post-hoc test)

day 9 when animals were tested after a saline injection. On the other hand, the ratio of conditioned changes was decreased by the higher doses of MDMA (Fig. 2b). Repeated measures ANOVA revealed a significant main effect of day of training [$F(3,105)=3.267$, $p<0.05$], dose [$F(4,35)=3.227$, $p<0.05$], without interaction between these two factors [$F(12,105)=1.802$, $p>0.05$]. To evaluate significant differences between doses, one-way ANOVAs were carried out for each day of training. MDMA at the dose of 30 mg/kg twice a day for 4 days significantly decreased the ratio of conditioned changes on all the sessions (days 5 to 8), and MDMA at the dose of 10 mg/kg reduced the ratio of conditioned changes on day 8 ($p<0.05$). However, these effects were no longer present when animals were tested on day 9 after a saline administration.

Figure 3 shows the time schedule for the different conditions followed in both studies where MDMA was administered sub-chronically (1, 3, 10 and 30 mg/kg twice daily for 4 days), before a training period in the shuttle boxes (Fig. 3a), and after a drug free training period (Fig. 3b).

Experiment 3: Effects of repeated MDMA pre-treatment on active avoidance acquisition and recall

Repeated treatment with MDMA before training induced slight effects in the number of conditioned changes, but when the ratio of conditioned changes was calculated, clear impairments were revealed in the acquisition of the active avoidance task, particularly in the late phase of training (Fig. 4). For conditioned changes, repeated measures ANOVA revealed a significant main effect of day of training [$F(12,480)=128.216$, $p<0.000$] but not significant effects of dose [$F(4,40)=0.913$, $p>0.05$], or in the interaction between these two factors [$F(48,480)=1.270$, $p>0.05$] were observed. Repeated measures ANOVA for the ratio of conditioned changes revealed a significant main effect of day [$F(9,378)=124.145$, $p<0.000$], dose [$F(4,42)=3.815$, $p<0.05$], and a significant interaction between these two factors [$F(36,378)=5.190$, $p<0.000$]. Post-hoc comparisons following significant

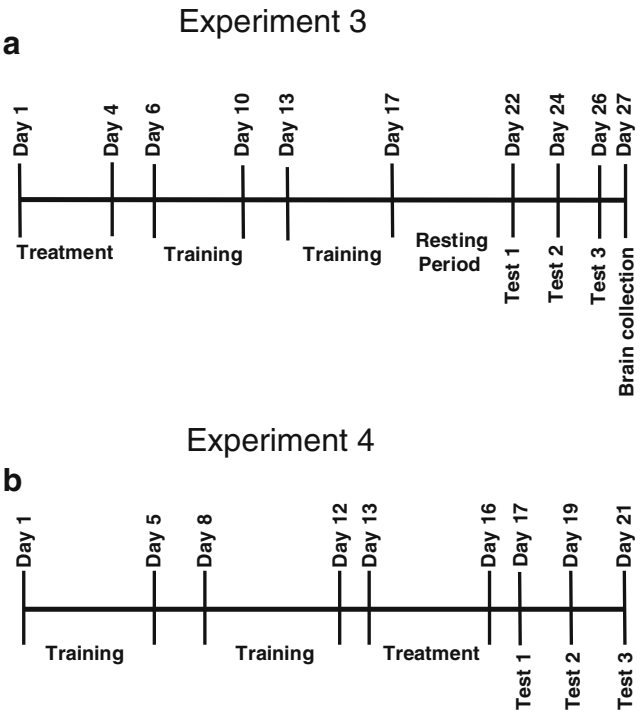

Fig. 3 Schematic representation of experiment 3 (**a**) and 4 (**b**) time schedules

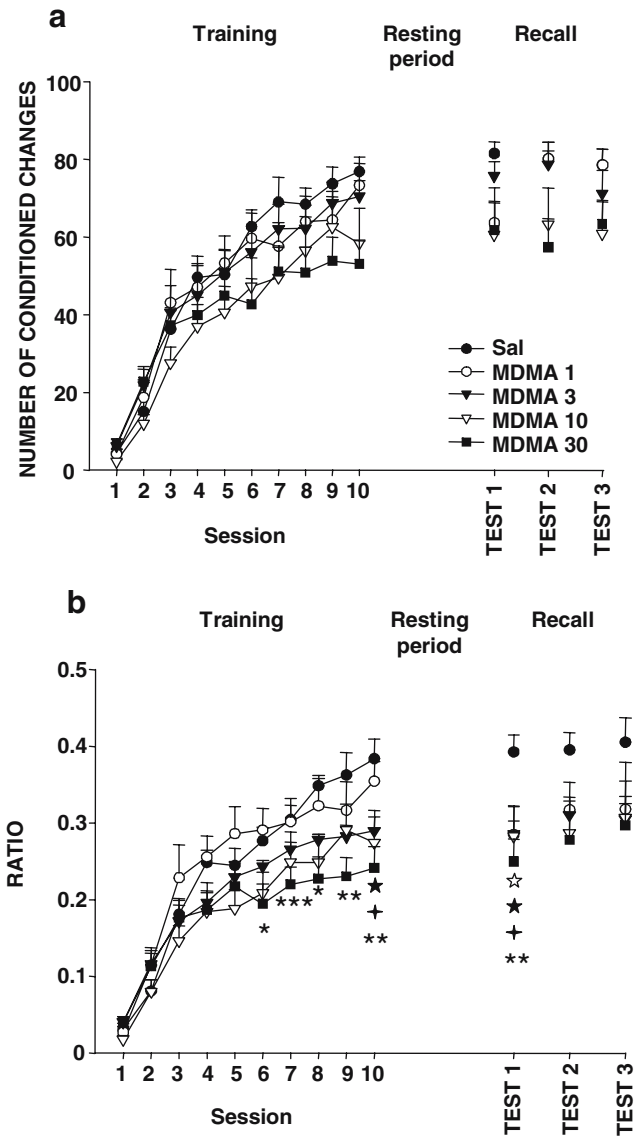

Fig. 4 Effects of repeated pre-treatment with MDMA on active avoidance acquisition and recall. Average number of conditioned changes (**a**) and ratio of conditioned changes (**b**) (+SEM) in the shuttle boxes in 100 trial sessions during 10 days of training following treatment with saline (n=9), MDMA 1 mg/kg (n=9), 3 mg/kg (n=6), 10 mg/kg (n=10) and 30 mg/kg (n=11) twice a day for 4 days. Following a 5-day resting period, all mice underwent three recall tests on alternate days. $p<0.05$ (MDMA 1 mg/kg vs saline); $p<0.05$ (MDMA 3 mg/kg vs saline); $p<0.05$ (MDMA 10 mg/kg vs saline); $p<0.05$, $p<0.01$, $p<0.001$ (MDMA 30 mg/kg vs saline, Dunnett post-hoc test)

one-way ANOVAs for each day of training revealed a significant decrease in the ratio of conditioned changes with respect to saline from days 6 ($p<0.05$) to 10 ($p<0.01$) in mice pre-treated with MDMA at the dose of 30 mg/kg. On day 10, mice treated with MDMA at the doses of 3 and 10 mg/kg also showed a decrease in the ratio of conditioned changes ($p<0.05$). More strikingly however, when animals were tested for recall of active avoidance 5 days after the last training session, all groups pre-treated with MDMA, even at the lowest dose, showed a reduced ratio of conditioned changes when compared to the saline group. During the following two recall tests, animals showed a tendency to re-learn the task, and no differences in the ratio of conditioned changes were revealed on the successive recall tests.

Experiment 4: Effects of repeated treatment with MDMA after training on active avoidance recall

Repeated treatment with MDMA at the dose of 30 mg/kg after a drug-free training period of 10 days interfered with the recall of the active avoidance task (Fig. 5a). No effects were revealed after the administration of lower doses of MDMA (Fig. 5). One-way ANOVA revealed a significant reduction in the number of conditioned changes only in the group of mice treated with the highest dose of MDMA (30 mg/kg) on the first recall test, 24 hours after the last day of treatment [$F(4,38)=3.184$, $p<0.05$]. On the following recall tests 2 and 3, no significant differences were observed

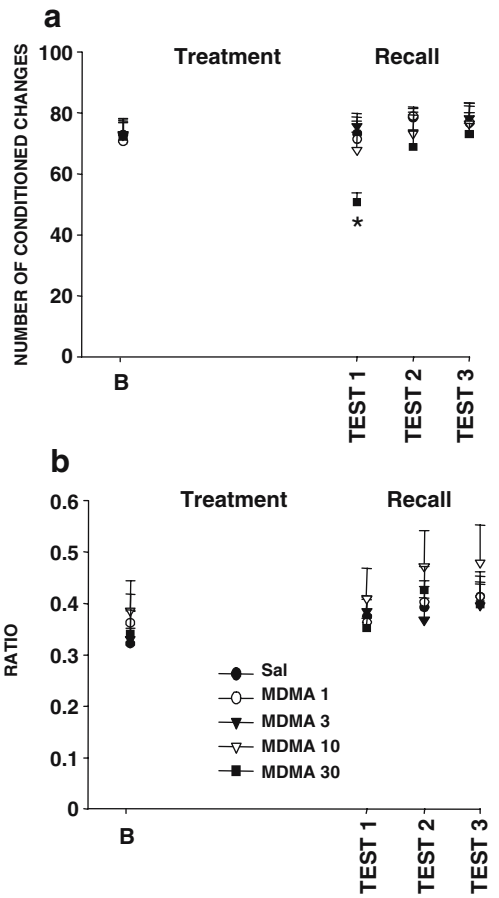

Fig. 5 Effects of a repeated treatment with MDMA on active avoidance recall. Average number of conditioned changes (**a**) and ratio of conditioned changes (**b**) +SEM in the shuttle boxes during baseline (B: the 3 last days of the training), and recall tests on alternate days after treatment with saline (n=8), MDMA 1 mg/kg (n=9), 3 mg/kg (n=7), 10 mg/kg (n=8) and 30 mg/kg (n=7) twice a day for 4 days. $p<0.05$ (MDMA 30 mg/kg vs saline, Dunnett post-hoc test)

between groups. For the ratio of conditioned changes with respect to total changes, no differences were revealed between the groups pre-treated with MDMA and the group pre-treated with saline in any recall test (Fig. 5b).

Autoradiography of [^{3}H] mazindol binding

To evaluate possible changes in presynaptic DAT binding sites, mice pre-treated with MDMA (30 mg/kg) or saline twice a day during 4 days were killed 4 days after the last day of treatment. In the group of animals treated with MDMA, the levels of [^{3}H] mazindol binding in the striatum (40.46±3.83 fmol/mg, n=14) were significantly lower than in the saline group (80.74±7.71 fmol/mg, n=10; p<0.001). An additional group of mice was used to evaluate the existence of enduring changes 23 days after the last MDMA (30 mg/kg) or saline administration (twice a day during 4 days). On this day, no significant differences in [^{3}H] mazindol binding levels in the striatum between the saline group (117.62±12.27 fmol/mg, n=6) and the group of mice treated with MDMA (120.70±14.11 fmol/mg, n=6) (p> 0.05) were observed. In a previous study, no changes in presynaptic DAT binding sites were observed using a lower dose of MDMA (10 mg/kg) in the same experimental conditions (Robledo et al. 2004).

Discussion

In this study, we show for the first time the effects of different dosing schedules of MDMA treatment on learning and recall of an active avoidance task in mice. Some of the deficits in learning and memory were produced at low doses and were not related to changes in striatal DAT binding sites.

Acute effects of MDMA during active avoidance acquisition

The acute administration of MDMA during the 4 days of training enhanced the performance of active avoidance in mice. However, this facilitating effect was state-dependent, as it was not revealed when mice were tested under drug-free conditions, except on day 5, where a significant impairment in the performance was observed only in mice previously receiving MDMA at 30 mg/kg twice a day for 4 days. The apparent enhancing effects of MDMA on active avoidance acquisition observed with 10 and 30 mg/kg (twice a day for 4 days) is possibly related to its locomotor activating effects because when conditioned learning was corrected for total activity (ratio of conditioned changes), these effects were no longer revealed. Animals treated with 3 mg/kg, however, performed better than controls, an effect that may be associated to the attention-enhancing properties of MDMA at this dose. Moreover, the acute administration of amphetamine, at the same dose and under the same experimental conditions, also induced a similar increase in the ratio of conditioned changes (data not shown), further supporting the implication of attention processes in the present results. In an experiment carried out in rats, it was also observed that the acute administration of MDMA at low doses (3.2 and 5.6 mg/kg) increased operant responding (Byrne et al. 2000). However, our results show that the higher rates of conditioned responses observed in these mice do not seem to sustain learning of the task, as the facilitation in active avoidance execution disappeared when MDMA was not administered on days 5 and 12.

In experiment 2, the effects of MDMA on performance of a previously learned task under drug-free conditions were evaluated. Mice treated with the dose of 10 mg/kg performed better than controls. A possible hyperlocomotor effect of MDMA can account for these results. Indeed, when conditioning was corrected for total activity, both 10 and 30 mg/kg of MDMA disrupted the correct execution of the previously learned task. The contribution of MDMA-induced anxiety in this effect is unlikely, as low doses of MDMA induce anxionegic-like effects, while high doses produce anxiolytic-like activity (Navarro and Maldonado 2002). In this experiment, the deleterious effects of high doses of MDMA were not observed on the test day after a saline injection, suggesting that MDMA disrupts performance of a previously learned task in a state-dependent manner. In this sense, a study carried out in monkeys showed that an acute administration of MDMA disrupted the behavioural performance in different tests of memory, and a return to baseline levels was observed after treatment (Taffe et al. 2001).

Although no studies on pain sensitivity have been conducted in mice, MDMA can induce antinociception in rats (Crisp et al. 1989). In our study, we cannot completely discard the contribution of the antinociceptive effects of MDMA in the results observed. However, when mice were trained after an injection of MDMA in experiment 1, no deficits in learning were observed with high doses, and enhanced learning was observed with the low dose, ruling out the contribution of changes in pain sensitivity in the deficits observed in experiments 1 and 2.

Effects of repeated MDMA pre-treatment on active avoidance acquisition and recall

Repeated pre-treatment with MDMA dose-dependently disrupted active avoidance learning and impaired the recall of this task, indicating that MDMA can have durable effects on cognitive processing. Thus, exposure to high doses of MDMA selectively impaired active avoidance acquisition, whereas the first recall test was impaired by both high and low doses of MDMA. A striking feature of the present results is that, in the

group of animals pre-treated repeatedly with MDMA at 30 mg/kg, the impairment in active avoidance learning was not evidenced until the sixth day of training, after a 2-day resting period, while no apparent effects were observed with lower doses until the last training session (tenth day of training). These results may reflect a dose-dependent effect of MDMA on recall mechanisms. Alternatively, it may be possible that MDMA does not disrupt early learning stages but that it dose-dependently affects later stages when a greater effort is required. After a 5 days resting period, the first recall test was disrupted in mice pre-treated with all doses of MDMA. These results show that learning impairments induced by higher doses of MDMA are persistent, but more remarkably, they also show that even low doses of MDMA, which do not affect learning, impair subsequent recall. Although the literature regarding cognitive deficits in ecstasy users is not straightforward, some studies report learning and memory impairments in regular users with respect to controls (Parrott et al. 1998; Morgan 1999; Morgan et al. 2002; Reneman et al. 2000; Rodgers 2000; Wareing et al. 2000; Zakzanis et al. 2007). Finally, our results show that the disruption in recall observed on the first test with all doses of MDMA recovers on subsequent tests days, suggesting that the effects of MDMA are clearly revealed when the requirements of the task increase, as is the case of the first recall session after a 5-day resting period. This recall test serves as a retraining period, and a progressive recovery takes place after this session. Therefore, a certain degree of recovery in performance is expected when using this experimental schedule.

Effects of a repeated treatment with MDMA on active avoidance recall.

To dissociate the effects of MDMA on learning and its effects on memory, recall of a previously learned task was evaluated during drug-free trials. This is an important issue because in most studies reporting memory impairments after MDMA, the drug was administered before training; thus, it is possible that these memory deficits also comprise acquisition deficits. In this study, we observed that mice treated with the high dose of MDMA (30 mg/kg) performed worse than controls in terms of the absolute number of conditioned trials on the first recall test, but when actual learning was corrected for total activity, this deficit was no longer present. This result is similar to the one observed in experiment 1, where mice treated with this dose of MDMA showed less conditioned changes than controls during session 5 after a saline injection, but no differences appeared in terms of the ratio of conditioned changes. Together, these findings indicate that high doses of MDMA producing strong locomotor activation may induce a subsequent state of motor quiescence, which is not related to previous experience of the task, as it appeared both in mice treated during and after training. The results of this experiment showing that MDMA does not disrupt recall of an active avoidance task, which has been learnt before treatment, can be contrasted with those observed in experiment 3, where all doses of MDMA disturb recall of a task learnt after treatment. Together, these results suggest that repeated medium and high doses of MDMA disrupt learning, and low doses prevent the consolidation an active avoidance task, both situations leading to recall deficits. However, once the task has been learnt and consolidated, MDMA does not impair recall mechanisms.

Reductions in DAT binding sites were found 4 days after the last treatment with MDMA (30 mg/kg), but this decrease was no longer present 23 days after the last injection, suggesting that the possible changes induced by MDMA on dopaminergic terminals in mice may recover over time. Although we cannot discard the possibility that the reduction in DAT binding observed 4 days after the last MDMA treatment (30 mg/kg) could account for the cognitive deficits, this possibility is unlikely, as deficits were also observed with low doses of MDMA not producing changes in DAT binding (Robledo et al. 2004). Diverse studies have established the importance of the dopaminergic system in the consolidation of different types of memory in mice (Castellano et al. 1991; Mele et al. 2004; Ferretti et al. 2005). Additionally, the relevance of the dopaminergic system on the acquisition and retention of spatial tasks has been demonstrated in rats with dopamine depletion (Whishaw and Dunnett 1985). In contrast to these data, our results show that learning and retention deficits can be obtained in mice without a parallel decrease in striatal DAT binding, further supporting previous findings in other species where impairments of sequential and spatial learning performance were reported after the exposure to MDMA with no important changes in brain region-specific dopamine, serotonin and norepinephrine levels (Broening et al. 2001).

In summary, acute MDMA was able to interfere with active avoidance acquisition and with the execution of a previously learned task in mice. In addition, repeated pre-treatment with MDMA reduced acquisition of active avoidance and impaired recall of this task. Importantly, we show that even at low doses, MDMA can produce deficits in the consolidation of new information.

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