CB₁ Cannabinoid Receptor Modulates 3,4-Methylenedioxymethamphetamine Acute Responses and Reinforcement

Clara Touriño, Catherine Ledent, Rafael Maldonado, and Olga Valverde

Background: 3,4-Methylenedioxymethamphetamine (MDMA) is a popular recreational drug widely abused by young people. The endocannabinoid system is involved in the addictive processes induced by different drugs of abuse. However, the role of this system in the pharmacological effects of MDMA has not yet been clarified.

Methods: Locomotion, body temperature, and anxiogenic-like responses were evaluated after acute MDMA administration in CB₁ cannabinoid receptor 1 knockout mice. Additionally, MDMA rewarding properties were investigated in the place conditioning and the intravenous self-administration paradigms. Extracellular levels of dopamine (DA) in the nucleus accumbens were also analyzed after a single administration of MDMA by in vivo microdialysis.

Results: Acute MDMA administration increased locomotor activity, body temperature, and anxiogenic-like responses in wild-type mice, but these responses were lower or abolished in knockout animals. 3,4-Methylenedioxymethamphetamine produced similar conditioned place preference and increased dopamine extracellular levels in the nucleus accumbens in both genotypes. Nevertheless, CB₁ knockout mice failed to self-administer MDMA at any of the doses used.

Conclusions: These results indicate that CB₁ cannabinoid receptors play an important role in the acute prototypical effects of MDMA and are essential in the acquisition of an operant behavior to self-administer this drug.

Key Words: Anxiety-like behavior, body temperature, conditioned place preference, intravenous self-administration, in vivo microdialysis, locomotion

The amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA or ecstasy) is a popular recreational drug with a particular mechanism of action, different from classical psychostimulants since it mainly acts on both dopamine (DA) and serotonin (5-HT) reuptake systems (1). In humans, MDMA produces euphoria, empathy, and increased energy, often accompanied by postdrug anxiety and agitation (2,3). The prototypical pharmacological responses induced by MDMA in animals include hyperthermia (4), hyperlocomotion (5), and anxiety-like responses (6,7). In addition, MDMA induces rewarding effects in different experimental models. Thus, this drug establishes conditioned place preference (CPP) in rats (8) and mice (9) and maintains operant intravenous self-administration in rats (10), mice (11), and monkeys (12,13). In agreement, MDMA administration increases DA extracellular levels in the nucleus accumbens, as revealed by in vivo microdialysis experiments (14).

In humans, MDMA is frequently consumed in combination with cannabis (15). Several interactions between MDMA and delta²-tetrahydrocannabinol (THC), the Cannabis sativa main psychoactive compound, have been reported. Some animal studies showed MDMA-THC interactions in pharmacological responses such as locomotor activity, body temperature, anxiety-like effects, and withdrawal syndrome (16,17). Delta²-tetrahydrocannabinol produces its psychoactive effects activating the CB₁ cannabinoid receptor 1, which is mainly expressed in the central nervous system (18). Several physiological responses that are mediated by MDMA administration, such as locomotor activity, body temperature, mood, learning, and reward, are also modulated by the endocannabinoid system (19,20). This system interacts with a variety of neurotransmitters, including DA and 5-HT (20,21), which are key components in the mechanism of action of MDMA. The endocannabinoid system also represents a common neurobiological substrate for the addictive properties of different drugs of abuse (22), including opiates (23), nicotine (24), ethanol (25), and cocaine (26).

In the present study, CB₁ receptor knockout mice were used to evaluate the role of this receptor in the acute pharmacological responses and reinforcing properties of MDMA.

Methods and Materials

Animals

Male CD1 (Charles River, L’Arbresle, France) CB₁ knockout mice on a CD1 background and their wild-type littermates, weighing 30 to 35 g at the beginning of the experiments, were used in this study (23). Mice were housed in a temperature (21° ± 1°C), humidity (55% ± 10%), and light-cycle controlled room. Food and water were available ad libitum. Light was on between 8:00 AM and 8:00 PM, and the experiments took place during the light phase. For the self-administration studies, mice were exposed to a reversed cycle, and the experiments took place during the dark phase. A different cohort of animals was used in each experiment. All animal care and experimental procedures were conducted according to the guidelines of the European Communities Directive 86/609/EEC regulating animal research and were approved by the local ethical committee (Comité Étic d’Experimentació Animal–Institut Municipal d’Assistència Sani-
Drugs

3,4-Methylenedioxyamphetamine hydrochloride was obtained from Lipomed, A.G. (Arlesheim, Switzerland), dissolved in 9% physiological saline, and injected in a volume of 0.1 mL/10 g body weight (intraperitoneal [IP] or intravenous [IV]). Ketamine hydrochloride (100 mg/kg; Imalgène 1000, Rhône Mérieux, Lyon, France) and xylazine hydrochloride (20 mg/kg; Sigma Chemical Co., Madrid, Spain) were mixed and dissolved in ethanol and water (1:9). This anesthetic mixture was injected in a volume of 0.2 mL/10 g body weight IP.

Acute Pharmacological Responses

Locomotor responses induced by MDMA were evaluated by using locomotor activity boxes (9 × 20 × 11 cm) (Imetronic, Lyon, France) in a low luminosity room (5 lux) with white noise, as previously reported (24). Locomotor activity was measured as the number of beam breaks on horizontal and vertical movements. Locomotor activity was recorded during 1 h immediately after MDMA (5, 10, 20, and 30 mg/kg IP) or saline injection in CB1 knockout mice and their wild-type littermates. To evaluate MDMA potency, locomotor activity was also measured under the same experimental conditions with MDMA (2.5, 5, 10, and 20 mg/kg IP or IV) or saline administration.

Body temperature was measured in each mouse by placing 3 cm of a thermocoupled flexible probe in the mice rectum for 10 sec (Panlab, Madrid, Spain). Basal temperature was measured 60 min before the injection. Temperature was also evaluated 30, 60, 90, 120, and 180 min after MDMA (10, 20, and 30 mg/kg) or saline injection in CD1 mice after MDMA (2.5, 5, 10, and 20 mg/kg IP or IV) or saline administration.

Place Conditioning Paradigm

The MDMA CPP was performed using a nonbiased procedure, as previously reported (9). The apparatus is composed of two main square conditioning compartments (15 × 15 × 15 cm) separated by a neutral area (29). During the conditioning phase, mice were pretreated with MDMA 10 mg/kg, IP or saline 30 min before being confined in the conditioning compartment for 30 min. Four pairings were carried out with MDMA and four pairings with saline on alternate days. A preference score was calculated for each animal as the difference between the time spent in the drug-paired compartment during the test and preconditioning phases.

Microdialysis Procedure

Microdialysis studies were performed as previously described (26). Three days after cannula guide implantation 1 mm above the nucleus accumbens (anteroposterior, +1.60 mm; mediolateral, −9 mm; dorsoventral, −3.60 mm from bregma), the analytical probe was inserted. Two days after probe implantation, animals were habituated to the experimental environment overnight. The following morning, Ringer’s solution was pumped through the dialysis probe at a constant rate of 1 μL/min. Four consecutive 20-min dialysis samples were collected to determine DA baseline levels. Then, mice were injected with either saline or MDMA (10 or 20 mg/kg) and samples were collected every 20 min for 4 hours. Dialyze samples (15 μL) were injected without any purification into a high-performance liquid chromatography (HPLC) system to quantify DA as previously reported (30).

At the end of the experiment, animals were killed, and brains were removed and stored at −80°C. Brains were sliced using a cryostat in 20 μm serial coronal sections, dyed with cresyl violet, and probe placement was checked under a microscope. Only those animals that had been implanted correctly were included in the study. To calculate AUC the following equation was used:

\[
AUC = \left[\sum_{n=1}^{N} \left(0.5 \times (T_n + T_{n+1}) \times t\right)\right] + \left[\sum_{n=1}^{N-1} \left(0.5 \times (T_n + T_{n+1}) \times t\right)\right]
\]

where \(T_n\) is the temperature increase values and \(t\) is the time (min) between the consecutive measurements.

Operant Self-Administration

The self-administration experiments were conducted as previously described (11). Operant responding was maintained by MDMA (0.03, 0.06, 0.125, and 0.25 mg/kg/infusion) delivered in 23.5 μL over 2 sec. Mice were trained to nose poke to receive an MDMA injection under a fixed ratio 1 schedule of reinforcement for 10 days. Self-administration sessions (2 hours daily) started with a priming infusion. The number of reinforcers was limited to 100 infusions per session and each reinforcer was followed by a 30-sec time-out period where active nose poking had no consequences. The stimulus light signaled delivery of the reinforcer. Mice were considered to acquire self-administration when they followed these three criteria for at least three consecutive sessions: 65% of the total number of responses were executed on the active hole, the number of responses on the active hole was higher than 5, and the mean deviation of the total number of reinforcers earned in three consecutive sessions was less than 30% (70% stability).

Statistical Analysis

Effects of MDMA on locomotor activity, anxiety-like behavior, CPP, and AUC values were compared by using a between-subjects two-way analysis of variance (ANOVA) (genotype and treatment), followed by one-way ANOVA for individual differences and post hoc comparisons (Dunnett’s test) when required. Body temperature and microdialysis data were analyzed using three-way ANOVA with treatment, genotype, and time factors. Three-way ANOVA with hole and genotype as between-group factors, and time as within-group factor, was used to analyze the acquisition of MDMA self-administration at the different training doses. Between-subjects two-way ANOVA with hole and genotype was calculated to compare the mean of nose pokes performed during the last 3 acquisition days, followed by one-way ANOVA when required. Unpaired two-tailed Student t test was used to compare the basal dopamine levels between genotypes. Paired Student t test was used to compare the time required to discriminate between active and inactive holes. Median effective dose (ED50) values to determine MDMA potency by IP or IV route were determined by nonlinear regression analysis.
of the dose-response curves using PRISM (GraphPad, San Diego, California). In all the experiments, differences were considered significant if the probability of error was less than 5%.

**Results**

**Comparative Potency of MDMA Administered by IP or IV Route**

3,4-Methylenedioxymethamphetamine (2.5, 5, 10, and 20 mg/kg) similarly increased locomotor activity when administered by IP or IV route. The ED$_{50}$ value (95% confidence limits) calculated for IP administered MDMA (5.61 mg/kg [4.35–6.86]) did not significantly differ from the ED$_{50}$ value calculated for IV administered MDMA (5.98 mg/kg [4.33–7.62]).

**MDMA Effects on Locomotor Activity**

3,4-Methylenedioxymethamphetamine (5, 10, 20, and 30 mg/kg) increased locomotor activity in a dose-dependent manner in both genotypes as revealed by two-way ANOVA (Table 1). However, this increase was significantly lower in CB$_1$ knockout animals (Figure 1), as indicated by one-way ANOVA (genotype), when MDMA was administered at the dose of 10 mg/kg [F(1,23) = 27.068, p < .001] and 20 mg/kg [F(1,19) = 15.646, p < .001] (Figure 1A). This attenuation was also observed for the vertical activity (rearing), since one-way ANOVA (genotype) revealed significant differences when MDMA was administered at the dose of 5 mg/kg [F(1,22) = 5.595, p < .05], 10 mg/kg [F(1,23) = 8.299, p < .01], 20 mg/kg [F(1,19) = 4.684, p < .05], and 30 mg/kg [F(1,22) = 6.478, p < .05] (Figure 1B).

**MDMA-Induced Hyperthermia**

3,4-Methylenedioxymethamphetamine (10, 20, and 30 mg/kg) induced hyperthermia in a dose-dependent manner in both genotypes. However, the elevation of body temperature was significantly lower in CB$_1$ knockout animals (Figure 2) (Table 2). Two-way ANOVA calculated for the body temperature of animals treated with MDMA 20 mg/kg showed a significant effect of genotype [F(1,17) = 9.577, p < .01], time [F(4,68) = 21.333, p < .001], and interaction between both factors [F(4,68) = 2.496, p = .05] (Figure 2C). Similarly, two-way ANOVA calculated for the body temperature of mice treated with MDMA 30 mg/kg revealed an effect of genotype [F(1,17) = 6.548, p < .05], time [F(4,58) = 8.877, p < .001], and interaction between both factors [F(4,68) = 2.519, p < .05]. No significant differences between genotypes were observed in animals treated with saline or MDMA 10 mg/kg. To compare the global effect of MDMA on body temperature, we calculated the AUC values for each dose evaluated (Figure 2E) (Table 1). One-way ANOVA (genotype) revealed significant differences between genotypes when MDMA was given at the dose of 20 mg/kg [F(1,20) = 10.994, p < .01] and 30 mg/kg [F(1,18) = 11.056, p < .05].

![Figure 1. Effects of acute MDMA (5, 10, 20 and 30 mg/kg, IP) on locomotion in CB$_1$ knockout (black bars) and wild-type mice (white bars). Horizontal (A) and vertical (B) locomotion were measured immediately after saline or MDMA treatment.](image)

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**Table 1. Two-Way ANOVA Calculated for MDMA Effects on Locomotor Activity, Body Temperature, Elevated Plus Maze, Conditioned Place Preference, and Self-Administration in CB$_1$ Knockout Mice**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>F-Value</th>
<th>p-Value</th>
<th>Genotype</th>
<th>F-Value</th>
<th>p-Value</th>
<th>Interaction</th>
<th>F-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizontal Activity</td>
<td>F(4,105) = 29.980</td>
<td>&lt;.001</td>
<td>F(1,105) = 14.648</td>
<td>&lt;.001</td>
<td>F(4,105) = 2.747</td>
<td>&lt;.05</td>
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<tr>
<td>Vertical Activity</td>
<td>F(4,105) = 17.252</td>
<td>&lt;.001</td>
<td>F(1,105) = 32.587</td>
<td>&lt;.001</td>
<td>F(4,105) = 3.055</td>
<td>&lt;.05</td>
<td></td>
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</tr>
<tr>
<td>Body Temperature (AUC)</td>
<td>F(3,77) = 23.021</td>
<td>&lt;.001</td>
<td>F(1,77) = 16.929</td>
<td>&lt;.001</td>
<td>F(3,77) = 1.733</td>
<td>ns</td>
<td></td>
<td></td>
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<tr>
<td>Elevated Plus Maze</td>
<td></td>
<td></td>
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<tr>
<td>% Time spent in the open arms</td>
<td>F(3,94) = 4.377</td>
<td>&lt;.01</td>
<td>F(1,94) = 3.177</td>
<td>ns</td>
<td>F(3,94) = 7.219</td>
<td>&lt;.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Entries in the open arms</td>
<td>F(3,94) = 11.891</td>
<td>&lt;.001</td>
<td>F(1,94) = 4.722</td>
<td>&lt;.05</td>
<td>F(3,94) = 1.315</td>
<td>ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conditioned Place Preference</td>
<td>F(1,75) = 15.323</td>
<td>&lt;.001</td>
<td>F(1,75) = .020</td>
<td>ns</td>
<td>F(1,75) = 3.499</td>
<td>ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self-Administration Dose-Response</td>
<td>F(3,69) = 1.776</td>
<td>ns</td>
<td>F(1,69) = 23.465</td>
<td>&lt;.001</td>
<td>F(3,69) = 3.248</td>
<td>&lt;.05</td>
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</table>

Two-way ANOVA with treatment and genotype as between-subject factors. Different doses of MDMA or saline were tested on locomotor activity (5, 10, 20, 30 mg/kg, body temperature (10, 20, 30 mg/kg), elevated plus maze (1, 10, 20 mg/kg), conditioned place preference (10 mg/kg), and operant self-administration (03, 06, 12, 25 mg/kg/infusion) in CB$_1$ knockout mice. See Methods and Materials for details.

ANOVA, analysis of variance; AUC, area under the curve; MDMA, 3,4-methylenedioxymethamphetamine; ns, nonsignificant.
Post hoc analysis showed a reduction in the percentage of entries in the open arms in wild-type animals when treated with MDMA at the dose of 10 mg/kg (p < .05) and 20 mg/kg (p < .001). A reduction in the percentage of entries in the open arms was also observed in knockout animals when treated with MDMA 20 mg/kg (p < .1). One-way ANOVA to compare both genotypes showed significant differences in the percentage of entries in the open arms between groups treated with saline [F(1,30) = 10.445, p < .01] and MDMA 1 mg/kg [F(1,30) = 7.425, p < .05] but not with MDMA 10 or 20 mg/kg (Figure 3B).

**MDMA-Induced Conditioned Place Preference**

3,4-Methylenedioxymethamphetamine induced similar rewarding effects in the CPP paradigm in both genotypes as revealed by two-way ANOVA (Table 1). The time spent in the drug-paired compartment during the preconditioning phase was similar in the different groups of animals [F(1,71) = .009, ns], ensuring the use of an unbiased procedure. One-way ANOVA showed a CPP in wild-type [F(1,37) = 4.319, p < .05] and knockout mice [F(1,34) = 10.394, p < .01] treated with MDMA (10 mg/kg) (Figure 4).

**Dopamine Extracellular Levels in the Nucleus Accumbens After MDMA Administration**

Basal extracellular DA levels in nucleus accumbens dialysates were similar in wild-type (8.1 ± 1.5 pg/15 μL) and knockout mice (6.9 ± 1.3 pg/15 μL) (p = .135, ns). 3,4-Methylenedioxymethamphetamine induced similar increase in DA extracellular levels in the nucleus accumbens in both genotypes as revealed by three-way ANOVA (Table 2). Acute MDMA at the dose of 20 mg/kg (IP) enhanced DA extracellular levels in both genotypes, whereas at the dose of 10 mg/kg did not produce any significant effect (Figure 5A).

Two-way ANOVA for AUC values (from 0 to 240 min) (Figure 5B) indicated a treatment effect [F(2,34) = 31.199, p < .001] without genotype effect or interaction between these two factors. One-way ANOVA (treatment) indicated an effect of MDMA in wild-type [F(2,17) = 9.247, p < .01] and knockout mice [F(2,17) = 29.287, p < .01], as well as post hoc analysis when MDMA was administered at 20 mg/kg (wild-type, p < .01; CB1 knockout, p < .001). The maximal MDMA-induced increase of DA levels was similar in both wild-type (318.9%) and knockout mice (391.1%) and occurred in both groups from 40 to 60 min after MDMA administration.

**MDMA Self-Administration**

Wild-type and knockout mice were trained to self-administer MDMA (0.3, 0.6, 12, and .25 mg/kg infusion) (Figures 6 and 7). 3,4-Methylenedioxymethamphetamine at the lowest dose (.03 mg/kg/infusion) did not maintain self-administration in any genotype, since no discrimination between the active and the inactive hole was observed (Figure 6A) (Table 2) and no animal reached the acquisition criteria. Only wild-type mice maintained a reliable MDMA self-administration at the doses of .06, 12, and .25 mg/kg/infusion (Table 2) (Figure 6B, 6C, and 6D). The percentage of wild-type mice that reached the acquisition criteria was 56% at the dose of .06 mg/kg/infusion, 67% at the dose of .12 mg/kg/infusion, and 57% at the dose of .25 mg/kg/infusion. No CB1 knockout mice trained with MDMA achieved the acquisition criteria at these doses. The time required to discriminate the active hole was MDMA dose-dependent. Thus, paired Student t test revealed that wild-type mice trained with MDMA .06 mg/kg/infusion discriminated between holes after 9 days of training (p < .05) (Figure 6B); animals receiving MDMA .12 mg/kg/infusion failed to achieve the acquisition criteria within 9 days.
mg/kg/infusion discriminated between holes after 5 days (p < .05) (Figure 6C); and animals self-administering MDMA .25 mg/kg/infusion discriminated between holes after 3 days (p < .5) (Figure 6D). 3,4-Methylenedioxymethamphetamine dose-response curve was calculated by the mean of infusions received in the last 3 days of the self-administration training (Figure 7). A bell-shaped curve was observed in wild-type mice, whereas no effect was revealed in knockout mice (Table 3). One-way ANOVA (genotype) showed significant differences between wild-type and knockout mice when MDMA was given at the dose of .06 mg/kg/infusion [F(1,16) = 9.307, p < .01], .12 mg/kg/infusion [F(1,22) = 16.086, p < .001], and .25 mg/kg/infusion [F(1,22) = 9.301, p < .01].

Discussion

This study demonstrates that CB1 cannabinoid receptors modulate several pharmacological responses and play a relevant role in the reinforcing effects of MDMA. Indeed, hyperlocomotion, hyperthermia, and anxiety-like behavior produced by MDMA were impaired in mice lacking CB1 cannabinoid receptors. Furthermore, CB1 knockout mice did not acquire self-administration behavior at any dose used of MDMA (.03, .06, .12, and .25 mg/kg/infusion), although this drug induced CPP and enhanced the extracellular levels of DA in the nucleus accumbens of mutant mice.

Hyperthermia is one of the most characteristic pharmacological effects of MDMA in humans and animals (32,33). 3,4-Methylenedioxymethamphetamine increases temperature by activating 5-HT receptors in the preoptic nucleus of hypothalamus (34), an area known to expresses CB1 receptors (35). No pharmacological studies have ever described the interaction between chronic administration of CB1 antagonist and MDMA. However, it has been reported that CP55,940 prevented MDMA-induced hyperthermia (16) and THC eliminated fluoxetine-induced hyperthermia (36). Then, the increase of 5-HT in the preoptic nucleus of the hypotal-

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Table 2. Three-Way ANOVA Calculated for MDMA Effects on Body Temperature and DA Release in CB1 Knockout Mice

<table>
<thead>
<tr>
<th></th>
<th>Body Temperature</th>
<th>In Vivo Microdialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F-Value</td>
<td>p-Value</td>
</tr>
<tr>
<td>Time</td>
<td>F(4,280) = 50.076</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Genotype</td>
<td>F(1,70) = 16.784</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Treatment</td>
<td>F(3,70) = 25.965</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Time × Genotype</td>
<td>F(4,280) = .937</td>
<td>ns</td>
</tr>
<tr>
<td>Time × Treatment</td>
<td>F(12,280) = 3.065</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Genotype × Treatment</td>
<td>F(3,70) = 1.525</td>
<td>ns</td>
</tr>
<tr>
<td>Time × Genotype × Treatment</td>
<td>F(12,280) = 2.490</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

Three-way ANOVA repeated measures with treatment and genotype as between-subject factors and time as within-subject factor. Different doses of MDMA or saline were tested on body temperature (10, 20, 30 mg/kg) and in vivo microdialysis (10 and 20 mg/kg). See Methods and Materials for details.

ANOVA, analysis of variance; DA, dopamine; MDMA, 3,4-methylenedioxymethamphetamine; ns, nonsignificant.

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Figure 3. Effects of acute MDMA (1, 10, and 20 mg/kg, IP) on anxiety-like responses in CB1 knockout (black bars) and wild-type mice (white bars). The behavioral responses in the elevated plus maze were measured 30 min after MDMA or saline administration. Data are expressed as mean ± SEM of (A) the percentage of time spent in the open arms and (B) the percentage of entries in the open arms in wild-type (n = 9 to 13) and CB1 knockout mice (n = 10 to 14). ***p < .001, **p < .01, *p < .05, ns p < .01, when compared with the saline group of the same genotype (Dunnett’s test). *p < .05, **p < .01, ***p < .001, when compared with the saline group of the same genotype. CB1 knockout mice did not acquire self-administration behavior at any dose used of MDMA (.03, .06, .12, and .25 mg/kg/infusion), although this drug induced CPP and enhanced the extracellular levels of DA in the nucleus accumbens of mutant mice.

Figure 4. Effects of saline and MDMA (10 mg/kg, IP) on the CPP paradigm in CB1 knockout (black bars) and wild-type mice (white bars). Four pairings were carried out with MDMA and four pairings with vehicle on alternate days (see Methods and Materials). MDMA and saline were administered 30 min before each conditioning session. Data are expressed as mean ± SEM of score values (test-pretest) in CB1 knockout (saline, n = 16; MDMA 10 mg/kg, n = 22) and wild-type mice (saline, n = 14; MDMA 10 mg/kg, n = 23). *p < .05, **p < .01 when compared with the saline group of the same genotype. CPP, conditioned place preference; IP, intraperitoneal; MDMA, 3,4-methylenedioxymethamphetamine; SEM, standard error of the mean.
amus that induces MDMA hyperthermia seems to be modulated by the cannabinoid system. Hyperlocomotion is a common response observed in animals after MDMA administration, and both DA and 5-HT systems participate in this effect (37). Previous studies have described a reduction of MDMA-induced hyperlocomotion by cannabinoid agonists in rats (16). The CB₁ receptor regulates motor activity, and is highly expressed in brain structures related with motor control (38). Interestingly, the hyperlocomotor effects of cocaine, which acts preferentially on DA reuptake, were not modified in CB₁ knockout mice (39). Therefore, the attenuation of MDMA-induced hyperactivity could be related to an interaction between CB₁ receptors and the 5-HT system rather than changes in DA functionality. Accordingly, different findings support the existence of cross-interactions between endocannabinoid and 5-HT system (40), particularly in the cerebellum (41) and the caudate-putamen (42), which are crucial brain areas in the control of motor coordination. However, the detailed nature of these interactions remains unclear.

Changes in anxiety-like responses are another prototypical effect of MDMA, although heterogeneous results have been reported depending on the dose, frequency of administration, and behavioral test used (1). This study reveals an anxiety-like effect after acute MDMA (10 mg/kg) administration in wild-type mice in accordance with previous studies performed under similar experimental conditions (6,7,43). The CB₁ knockout mice used in this study presented an anxiety-like phenotype, as previously reported (43-45). However, MDMA did not modify the anxiety-like behavior of mutant animals in the elevated plus maze. Previous studies have suggested an interaction between MDMA and the cannabinoid system in emotional responses. In this sense, THC reduced the anxiety-like effects of MDMA (5 mg/kg) in the emergence test in rats (16). This interaction could also be related to the modulation of 5-HT transmission by the cannabinoid system, since the anxiolytic-like effect of the 5-HT₁A agonist buspirone was lower in CB₁ knockout mice (45).

3,4-Methylenedioxymethamphetamine rewarding properties have been widely described in previous studies (9-13). The blockade of CB₁ receptor with rimonabant abolished MDMA self-administration (46) and MDMA-induced CPP in rats (47). These results are in contrast to our study where no differences between genotypes were revealed in MDMA-induced CPP. Differences in the species, route of administration, and experimental conditions used in these studies could explain the differences. In the present study, MDMA administration produced a similar enhancement of DA extracellular
levels in the nucleus accumbens in both genotypes. In contrast, MDMA self-administration was abolished in CB1 mutant mice. A possible learning impairment can be excluded to explain the lack of self-administration in CB1 knockout mice, since these animals exhibited similar operant behavior maintained by natural stimuli such as food and water (26). The absence of MDMA self-administration and the presence of MDMA-induced CPP in these mutant mice can be explained by the different behavioral parameters evaluated in these paradigms and the different neural pathways involved in each task performance. Operant self-administration is a contingent model in which the drug is freely self-administered. Nevertheless, the drug is passively received by animals in CPP. On a contingent operant paradigm such as self-administration, multiple brain circuits involved in motivation, consciousness, and long-term learning are also engaged, while CPP mainly evaluates the effect of a drug on the circuits of primary reward, namely, the mesolimbic system. Thus, mediodorsal thalamus and prefrontal cortex, which participate in consciousness and motivation, show increased cerebral metabolic rates in psychostimulant self-administering animals but not in animals with passive drug administration such as CPP (48). Then, CB1 receptor seems to play a specific role in these brain circuits that control complex operant responses but not in the circuits of MDMA primary reward. The CB1 cannabinoid receptor is essential in the acute rewarding effects of several drugs of abuse that produce their effect on ventral tegmental area, such as nicotine, ethanol, or morphine (22). However, psychostimulant drugs produce their effect directly on the nucleus accumbens DA terminals. A low percentage of CB1 knockout mice (25%) acquired cocaine (49,50) could justify this difference in acquisition. However, the reinforcing efficacy of MDMA with regard to cocaine self-administration (26), whereas no animal trained with MDMA did. The lower reinforcing efficacy of MDMA with regard to cocaine (49,50) could justify this difference in acquisition. However, the role that the 5-HT system plays in MDMA but not in cocaine reinforcing properties seems to be the key for the complete elimination of MDMA self-administration in CB1 knockout mice (51-53). The relevance of 5-HT in MDMA reinforcing properties suggests an involvement of the cortical neurons activated by this neurotransmitter in the abolishment of operant self-administration in CB1 knockout mice. Glutamatergic projections from prefrontal cortex to the mesolimbic system control motivation and the initiation of the appropriate responses to seek for a rewarding stimulus (54). These glutamatergic neurons express 5-HT receptors in the cell bodies of the prefrontal cortex (55) and CB1 receptors in the nucleus accumbens terminals (56). In addition, these pyramidal neurons show reduced dendritic arborization in CB1 mutant mice (57). Thus,

Figure 6. Acquisition of MDMA self-administration behavior in CB1 knockout (black) and wild-type mice (white). Data are expressed as mean ± SEM of the number of nose pokes in the active and the inactive holes in the 2-hour sessions performed during 10 days at (A) .03 mg/kg/infusion (n = 4 to 7); (B) .06 mg/kg/infusion (n = 9); (C) .12 mg/kg/infusion (n = 11 to 13); and (D) .25 mg/kg/infusion (n = 10 to 14). * p < .05; ** p < .01 comparison between holes (one-way ANOVA). ANOVA, analysis of variance; MDMA, 3,4-methylenedioxyamphetamine; SEM, standard error of the mean.

Figure 7. Dose-response curve for MDMA self-administration in CB1 knockout (black) (.03 mg/kg/infusion, n = 7; .06 mg/kg/infusion, n = 9; .12 mg/kg/infusion, n = 11; .25 mg/kg/infusion, n = 10) and wild-type mice (white) (.03 mg/kg/infusion, n = 4; .06 mg/kg/infusion, n = 9; .12 mg/kg/infusion, n = 13; .25 mg/kg/infusion, n = 14). Figure represents the mean of infusions of the last 3 days of acquisition at the different MDMA doses used. Data are expressed as mean ± SEM. * p < .05; ** p < .01; *** p < .001 comparisons between genotypes (two-way ANOVA). ANOVA, analysis of variance; MDMA, 3,4-methylenedioxyamphetamine; SEM, standard error of the mean.
the 5-HT activation in glutamatergic projections from prefrontal cortex to mesolimbic areas produced by MDMA (58) could be impaired in CB1 knockout mice, since these pyramidal neurons are modulated by CB1 receptors (56).

In summary, this study shows that CB1 receptor mediates the prototypical pharmacological responses induced by acute MDMA. This receptor is also critical for the acquisition of an operant behavior to self-administer MDMA through the modulation of neuronal circuits related to reward, motivation, and long-term learning. However, CB1 receptor does not participate in the acute rewarding properties of MDMA revealed in a noncontingent paradigm in which DA transmission would play a predominant role. This study provides additional findings to support the crucial role played by the endocannabinoid system in the control of the addictive behavior. The CB1 receptors directly participate in the rewarding properties of different drugs of abuse including cannabinoids, opioids, alcohol, and nicotine (22) but are also required for the acquisition of cocaine (26) and MDMA self-administration. 3,4-Methylenedioxymethamphetamine has a low addictive potential, but important health risks are associated with the consumption of this drug, such as hyperthermia, mood alterations, and binge administration, which could also be minimized by the chronic blockade of CB1 cannabinoid receptors.

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There are no conflicts of interests for any of the authors relating to these results.


