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# MDMA (ecstasy) effects in pubescent rats: Males are more sensitive than females

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**Abstract**

In Experiment 1, we assessed the effects of 3,4-methylenedioxymethamphetamine (MDMA) on locomotor activity in pubescent male and female Long–Evans rats. Thirty-nine day old rats were injected ip with 10 mg/kg of MDMA (ambient temperature 25 °C) three times at 2 h intervals. Initially, females showed greater locomotor activation by the drug than males, however after the second injection, males showed greater hyperlocomotion. After the third injection, 3 of 10 females and all of the males died. In the surviving females, we observed serotonin depletion in cortex and hippocampus, but catecholaminergic markers were unaltered. In Experiment 2, male and female rats were repeatedly injected with saline or 2, 5 or 10 mg/kg MDMA and body temperature was measured (ambient temperature 21.5 °C). After the third injection of 10 mg/kg MDMA, the MDMA-induced hyperthermia was greater in males than in females (about +0.8 °C); at the lower dose, no difference was observed. Probably because of the lower ambient temperature, only 1 female and 2 males succumbed to the MDMA treatment, and MDMA induced less serotonin depletion than in the first experiment, with no difference between females and males. Thus, pubescent males appear to be more sensitive than females to locomotor and hyperpyretic effects of MDMA. This sex-dependent effect, which is at variance with previously reported dimorphisms in psychostimulant effects, is discussed in terms of possible differences in dopamine $D_1$ and $D_2$ receptors at pubescence, or other factors related to drug metabolism.


*Keywords:* Ecstasy; Lethality; Locomotor activity; MDMA; Pubescence; Sexual dimorphism

## 1. Introduction

Psychostimulants like amphetamine, cocaine, or the increasingly popular recreational drug 3,4-methylenedioxymethamphetamine (MDMA or ecstasy) produce various physiological and behavioral effects, including locomotor hyperactivity (e.g., Green et al., 2003). Locomotor activity is usually, though not exclusively, linked to functions in dopaminergic systems of the midbrain, particularly in the nucleus accumbens (e.g., Weiner et al., 1996). Reports in the literature show a sexual dimorphism in the responsiveness of these systems to cocaine, D-amphetamine (e.g., Battaglia et al., 1988; Bisagno et al., 2003; Cailhol and Mormede, 1999), or dopaminergic ligands (Heijtz et al., 2002; Schindler and Carmona, 2002). Concerning MDMA, the question of a possible sexual dimorphism has not been addressed very thoroughly; however, in humans, women might be more susceptible than men to neurotoxic effects of MDMA (Reneman et al., 2001). Among demographic studies, some provide data on sex ratio among MDMA users (e.g., Pedersen and Skrondal, 1999; Riley et al., 2001; Simon and Mattick, 2002). This ratio may vary from less than 1:1 to 3:1 (male/female), depending on many factors (populations studied, selection of sample populations,

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age...). In a recent study concerning MDMA-related deaths in England and Wales (Schifano, 2004), the reported sex ratio for lethality was 4:1, males/females. As this ratio exceeds the largest reported in consumers, and although extreme caution is required as females and males manifest clearly different consumption patterns (Winstock et al., 2001), it may be questioned whether females are more or less sensitive to MDMA than males. In mice, females are more resistant to the toxic effects of MDMA than males: at a dose of 80 mg/kg, about 10% of males survive vs. 50% of females (Cadet et al., 1994). In another study (Miller and O'Callaghan, 1995), 20 mg/kg MDMA killed 4 out of 6 male mice, but no female. A potential problem with the mouse model, is that, in mice, MDMA produces dopamine but apparently no serotonin depletion, while opposite effects are seen in rats and primates, including humans (Green et al., 2003). Thus, our first experiment for this study was performed in rats, primarily to assess sex differences in the effects of MDMA on locomotor activity as an index of drug sensitivity. Male and female rats were given three i.p. injections of MDMA (10 mg/kg) every 2 h and activity was monitored over 6 h. This treatment regimen is similar to those used in recent studies, in adult (e.g., citations by Green et al., 2003) and adolescent rodents (e.g., Piper and Meyer, 2004). So far, only a few studies have addressed the effects of MDMA in pubescent rodents (e.g., Morley-Fletcher et al., 2002, 2004), and such experiments may be of interest regarding that a large proportion of those taking the drug are adolescents. In our first experiment (see below) males evinced higher locomotor activity and lethality than females. Following on these findings, we designed a second experiment to test whether sex differences in the hyperpyretic effects of MDMA injections might be a possible etiological factor in lethality.

## 2. Experiment 1

### 2.1. Materials and methods

#### 2.1.1. Subjects

All procedures were conducted in conformity with National and International Institutional Animal Care and Use Guidelines (Council Directive 87848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale; permission 6212 to J-C.C. and 6714-bis to H.J.; NIH publication, 86-23, revised 1985). This first experiment used 40 Long–Evans rats (C.E.R. Janvier, St-Berthevin, France), 20 females (weighing 112.6±1.6 g right before the first injection) and 20 males (weighing 136.3±1.6 g), delivered to the laboratory at the age of 30 days (PND 30). They were housed individually in transparent Makrolon cages (42×26×15 cm) under a 12:12 h dark/light cycle (lights on at 7.00 a.m.) with food and water available ad libitum. The vivarium was kept at 23±1 °C.

#### 2.1.2. Locomotor activity and drug treatment

Ten males and ten females were randomly assigned to a group receiving D,L-MDMA hydrochloride (Euromedex, Strasbourg, France; 10 mg/2 ml/kg, i.p.); the controls were injected with 0.9% NaCl (2 ml/kg). Three days before starting drug treatment (PND 36), all rats were brought to the experimental room where locomotor activity in their home cage was recorded over 24 h. Each cage (8 cages/shelf; 2×4 shelves in the room) was traversed by two infrared light beams targeted on two photocells, 4.5 cm above floor level and 28 cm apart. There were 8 cages per shelf and the rats were placed on each shelf so as to alternate males and females and randomise MDMA and NaCl injections. The number of cage crossings (successive beam interruptions) was monitored continuously by a computer in 10-min intervals. At PND 39, each rat was given three injections of MDMA (10 mg/kg each) or NaCl. Injections occurred 2 h apart. All rats were injected with the same drug solution. Dose and inter-injection intervals were chosen based on the literature (Broening et al., 1995; Fone et al., 2002; Morley-Fletcher et al., 2002, 2004; Piper and Meyer, 2004; see also other references in Green et al., 2003) and preliminary experiments in adolescent rats (Koenig and Cassel, unpublished) showing that a single injection of 10 mg/kg induced a hyperactivity which had returned to near-normal levels after about 120 min. Activity measures were made at an ambient temperature of 25 °C.

#### 2.1.3. Determination of monoamine concentrations

Thirteen days after the injections, all female rats (that survived the third MDMA injection—see results below) and their controls (i.e., 10 treated with NaCl and 7 treated with MDMA) were sacrificed by microwave irradiation (2.0 s; 6.3 kW; Sairem, Villeurbanne, France). After decapitation, the brain was extracted and dissected on a cold plate into prefrontal, frontal, temporal and occipital cortices, and both hippocampi were separated into a dorsal (septal pole) and a ventral (temporal pole) portion. The left and right structures from each rat were pooled, weighed and kept at −80 °C until neurochemical determination. The concentrations of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), noradrenaline (NA), serotonin (5-HT) and 5-hydroxyindolacetic acid (5-HIAA) were measured using high performance liquid chromatography (HPLC) with electrochemical detection. The tissue samples were prepared for HPLC by homogenization in 1 N formic acid/acetone (18:8.5, vol/vol), and the formic extracts were used for monoamine determinations. The monoamine concentrations were measured without further purification. The HPLC system consisted of an ESA liquid chromatography pump (ESA Inc., Bedford) coupled to an ESA Coulochem II detector (Eurosep Instruments) equipped with a 5014 high performance analytic cell (ESA Inc., Bedford). The detector potential at the analytic cell was set at +0.4 V. High performance liquid chromatography analysis was performed on a C18 Spherisorb ODS2

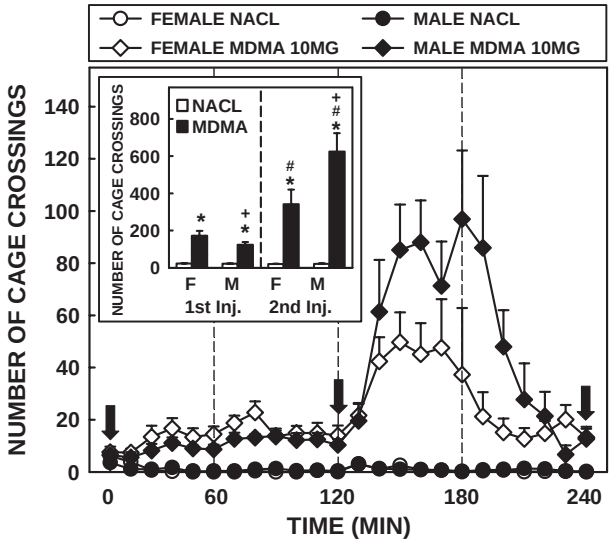


Fig. 1. Average locomotor scores (mean ± S.E.M.) recorded in the home cage in 10-min intervals from right after the 1st injection to right before the 3rd injection in female (F) and male (M) rats subjected to control (NACL) or MDMA injections. The times at which injections were made are indicated by the arrows (right above the highest curves). Bars in the insert correspond to the total activity recorded over the 2-h blocks that followed the 1st (left) or the 2nd injection (right). Given that after the 3rd injection 30% female rats and all male rats died (see Fig. 2), analysis of the subsequent locomotor scores in the remainders did not appear worthwhile. Statistics in the insert: *significantly different from NaCl in the corresponding post-injection time window (first 2 or last 2 h), $p<0.05$ (Newman–Keuls after ANOVA); #significantly different from the scores found after the first injection within the same sex and drug conditions, $p<0.05$ (paired $t$-test); +significantly different from females in the corresponding post-injection time window, $p<0.05$ (Newman–Keuls after ANOVA).

reverse phase column (5 μm pore size, 4.6 mm in diameter, 25 cm long). The mobile phase consisted of 0.1 M $NaH_2PO_4$, pH = 3, containing 0.1 mM/l of EDTA, 1.7 mM/l 1-octane sulfonic acid sodium salt and 10% acetonitrile. The flow rate was 1 ml/min. Concentrations of the different compounds were determined with a data analysis software (Baseline 810, Waters) and were expressed as ng/mg microwaved tissue.

### 2.1.4. Statistical analysis

Because 3 females and all 10 males given MDMA died after the 3rd injection, only behavioral data recorded after the first two injections were analyzed. Statistical analysis of locomotor activity scores and neurochemical data used analysis of variance followed by Newman–Keuls tests or paired $t$-test where appropriate (Howell, 1997). A $Chi^2$-test was used to compare death rates in females and males after the 3rd MDMA injection.

## 2.2. Results

### 2.2.1. Locomotor activity

Nocturnal and diurnal activity was not different among the four groups prior to injections (not illustrated). Locomotor activity scores recorded after the two first injections are presented in Fig. 1. The curves show the activity changes over successive 10-min intervals. In order to simplify an analysis that would require four factors to be considered (Sex, Drug, Injection, Intervals), we considered the total activity in 2-h blocks (insert in Fig. 1). The curve is nevertheless presented to illustrate activity changes over time. Regarding the fact that the variability after the second MDMA injection was much greater than after the first, ANOVAs of the overall scores recorded after each injection were performed separately; one considered the total activity recorded over the 2 h that followed the first injection, and another, the total activity recorded over the 2 h that followed the second injection (see Fig. 1, insert). The activity scores found after each injection in each sex and drug group were compared using a $t$-test for paired samples. After the first injection, there was a significant overall Drug effect ($F$ 1/36 = 81.6, $p<0.001$), rats given MDMA being significantly more active than those given NaCl; there was neither a significant overall Sex effect ($F$ 1/36 = 2.7, $p>0.10$), nor a significant interaction between factors Sex and Injection ($F$ 1/36 = 2.5, $p>0.10$). Interestingly, post hoc Newman–Keuls comparisons showed that MDMA-treated females were significantly more active than males ($p<0.05$), while the activity of females and males given NaCl was not significantly different from each other. After the second injection, there were significant effects for Sex ($F$ 1/36 = 5.0, $p<0.05$), Drug ($F$ 1/36 = 55.5) and Sex × Drug interaction ($F$ 1/36 = 5.0, $p<0.05$). Overall, MDMA caused sustained hyperactivity, and males were significantly more affected than females. The interaction was derived from the greater increase from saline seen in males.

### 2.2.2. Lethality following the third injection

The number of rats that survived the third injection is illustrated in Fig. 2. Seven out of ten females, but no male, survived this injection. This difference was significant ($Chi^2 = 10.77$, $p<0.001$).

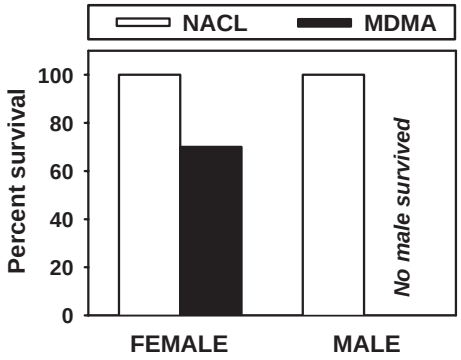


Fig. 2. Number of rats surviving the third MDMA injection (10 mg/kg, i.p.). F: females; M: males. Each group comprised 10 rats at the start of the experiment.

*2.2.3. Neurochemical measures in females*

All data are shown in Table 1. ANOVA for concentrations of DA, DOPAC and noradrenaline failed to show a significant treatment effect in all structures examined. Conversely, the concentration of serotonin was reduced significantly by MDMA in the prefrontal ($F$ 1/15=7.2, $p<0.05$), frontal ($F$ 1/15=11.5, $p<0.01$), temporal ($F$ 1/15=7.6, $p<0.05$) and occipital ($F$ 1/15=10.3, $p<0.01$) cortices, as well as in the dorsal ($F$ 1/15=17.7, $p<0.001$) and ventral ($F$ 1/15=23.2, $p<0.001$) hippocampus. A similar pattern was seen for concentrations of 5-HIAA, except that, in the dorsal hippocampus, the MDMA-induced reduction was not significant ($F$ 1/15=3.1, $p=0.10$).

*2.3. Discussion of Experiment 1*

The present results demonstrate clear-cut sex-related differences in the response of pubescent rats to initial and repeated treatment with MDMA. Initially, MDMA induced hyperlocomotion in both sexes, with females showing a greater initial response. The second injection produced a markedly increased hyperlocomotion in both sexes but this time, the males were significantly more activated. Quite unexpectedly, the third injection of MDMA killed 3 out of 10 females, but all of the males. In the females which survived the third injection, we found serotonin to be reduced in both the hippocampus and the cortex. This observation is in line with the literature (Sabol et al., 1996).

The differential sensitivity of males and females cannot be explained by (even subtle) differences in experimental conditions, as males and females were tested at the same time, in the same room and with the same drug solution. These results are consistent, however, with the results of two studies using mice (Cadet et al., 1994; Miller and O'Callaghan, 1995), although the aim of the latter studies was not to address the question of sex effects in MDMA-related lethality. Given the possible role of hyperthermia in MDMA fatalities and also serotonin depletion (Green et al., 2003), we designed a second experiment in which the hyperpyretic effects of MDMA were assessed in pubescent males and females. Because the magnitude of the MDMA-induced hyperthermia depends on the ambient temperature (O'Loinsigh et al., 2001), we conducted this second experiment at a lower temperature (21.5 °C instead of 25

Table 1
Concentration of NA, DA, DOPAC, 5-HT and 5-HIAA in various brain structures of female rats subjected to MDMA ($n=7$) or control (NACL; $n=10$) injections in Experiment 1

| | NA | DA | DOPAC | 5-HT | 5-HIAA |
|---|---|---|---|---|---|
| *Prefrontal cortex* | | | | | |
| NACL | 93.6±6.6 | 398.2±160.2 | 98.2±26.1 | 299.7±30.5 | 170.6±18.6 |
| MDMA | 79.2±8.6 | 340.7±90.2 | 82.9±9.8 | 168.0±23.8* | 105.4±14.8* |
| % Change | −15.4 | −14.5 | −15.6 | −43.9 | −38.2 |
| *Frontal cortex* | | | | | |
| NACL | 66.9±3.8 | 157.5±17.1 | 45.6±6.6 | 192.8±10.6 | 135.0±8.2 |
| MDMA | 67.6±2.9 | 153.8±24.3 | 47.4±5.4 | 127.9±11.4* | 84.4±6.7* |
| % Change | +0.1 | −2.4 | +3.9 | −33.7 | −37.5 |
| *Temporal cortex* | | | | | |
| NACL | 103.4±10.9 | 131.9±30.6 | 31.7±4.7 | 255.9±25.6 | 156.5±17.8 |
| MDMA | 106.9±10.9 | 147.0±42.6 | 27.9±2.1 | 144.3±19.3* | 88.8±15.2* |
| % Change | +3.3 | +11.4 | −12.0 | −43.6 | −43.3 |
| *Occipital cortex* | | | | | |
| NACL | 62.9±3.6 | 18.8±2.2 | 8.2±0.9 | 163.8±7.6 | 99.1±4.3 |
| MDMA | 62.1±7.2 | 13.8±2.8 | 6.7±0.5 | 102.0±17.9* | 49.0±12.7* |
| % Change | −0.1 | −26.6 | −18.3 | −37.7 | −50.6 |
| *Dorsal hippocampus* | | | | | |
| NACL | 80.4±6.7 | 23.9±3.3 | 9.4±1.4 | 247.4±13.7 | 383.0±111 |
| MDMA | 75.1±10.4 | 16.4±1.7 | 9.4±1.2 | 134.1±21.4* | 144.3±25.9* |
| % Change | −6.6 | −31.4 | 0.0 | −45.8 | −62.3 |
| *Ventral hippocampus* | | | | | |
| NACL | 120.2±6.1 | 22.4±1.6 | 9.9±0.4 | 322.3±19.5 | 275.0±15.1 |
| MDMA | 126.1±10.0 | 20.6±1.9 | 9.9±0.6 | 179.2±23.1* | 144.1±21.5* |
| % Change | +4.9 | −8.0 | 0.0 | −44.4 | −47.6 |

Rats were injected at 3 occasions, 2 h apart. All data are in pg/mg irradiated tissue.
*Statistics*: *$p<0.05$ vs. NaCl group.

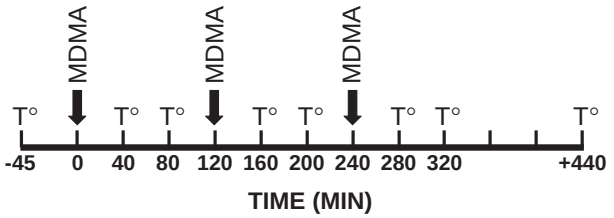


Fig. 3. Schematic representation of the protocol of our second experiment. Temperature measurements are indicated by *T*°. Injections of MDMA are indicated by black arrows.

°C in Experiment 1). We also used lower doses of MDMA in Experiment 2.

## 3. Experiment 2

### 3.1. Materials and methods

#### 3.1.1. Subjects

This experiment used 54 Long–Evans rats (C.E.R. Janvier, St-Berthevin, France), 27 females (116.2 g ± 1.3 g, when given the first injection, see below) and 27 males (134.3 ± 1.2 g), delivered to the laboratory at PND 30. They were housed as in Experiment 1. The colony room was kept at 23 °C.

#### 3.1.2. Drug treatment and temperature measurements

The injection and temperature measurement protocol is illustrated in Fig. 3. The ambient temperature in the experimental room was 21.5 °C. Five days before starting the temperature measurements, all rats were brought to the experimental room. On each of the 2 days preceding drug injections (PND 37, PND38), the body temperature of all rats was measured in order to habituate them to the measurement protocol. Rectal temperature was measured with a Pic indolor Vedo Flex (Artsana-Grandate, Italy) digital thermometer with a 0.1 °C precision, and lubricated with medical Vaseline. Determination of the temperature took a maximum of 30 s. On the drug injection day (age PND39), the first measurement was taken 45 min before drug treatment (between 10:30 and 11:15 a.m.). Each rat was injected at three occasions with saline or 2, 5 and 10 mg/kg MDMA (ip, 2 ml/kg; corresponding group abbreviations: NACL, MDMA 2MG, MDMA 5MG and MDMA 10MG, respectively). Injections were separated by 2 h. The other measurements were made 40, 80, 160, 200, 280, 320 and 440 min after the first drug administration. Between measurements, the rats remained in their home cage. With the exception of the occasions of temperature measurements, rats had free access to food and water. Observation of the rats' behavior in their home cage was possible between each series of temperature measurements. Six males and six females received saline injections. For each dose of MDMA, seven males and seven females were used.

#### 3.1.3. Determination of monoamine concentrations

This determination was made exactly as in Experiment 1 in the six and five surviving females and males, respectively.

#### 3.1.4. Statistical analysis

Statistical analysis of temperatures used a Sex (male, female) × Dose (0, 2, 5, 10 mg/kg) × Injection (1st, 2nd, 3rd) ANOVA, followed by Newman–Keuls tests or paired *t*-test where appropriate (Howell, 1997). Analysis of neurochemical data used a Sex × Dose design.

### 3.2. Results

#### 3.2.1. Temperature measurements

Data are shown in Fig. 4. The ANOVA failed to show a significant overall Sex effect ($F$ 1/46 < 1.0), but there was a significant Dose effect ($F$ 3/46 = 22.1, $p < 0.001$) as well as a significant Injection effect ($F$ 7/322 = 35.8, $p < 0.001$). The significant Dose effect was due to overall temperatures that were significantly higher at 5 and 10 mg/kg as compared to NACL or MDMA at 2 mg/kg ($p < 0.05$ at least). Overall temperatures after NACL injections were not significantly different from those found after MDMA at 2 mg/kg. Temperatures after 10 mg/kg were significantly higher than after MDMA at 5 mg/kg. The significant Injection effect

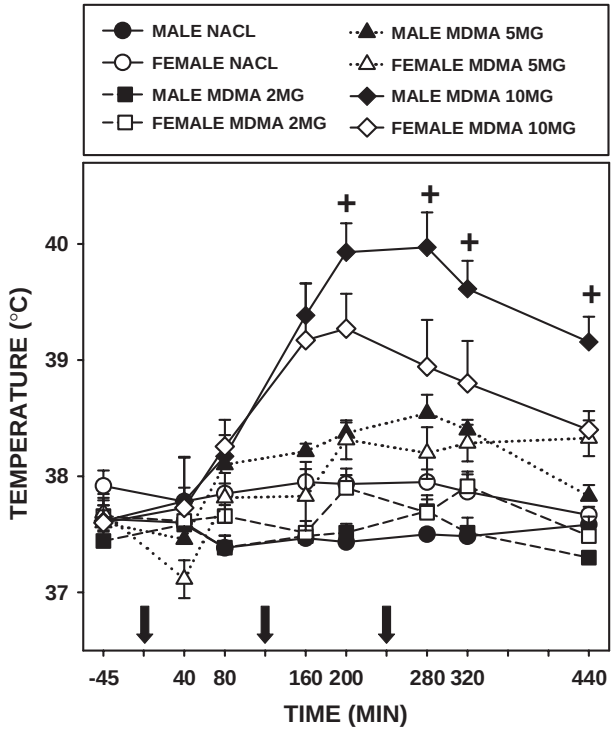


Fig. 4. Mean body temperature (mean + S.E.M.) at various delays (40, 80, 160, 200, 280, 320 and 440 min) after cumulative injections of saline (NACL; injection volume 2 ml/kg) or one of three doses of (±)-3,4-methylenedioxymethamphetamine (MDMA). Injections were made on three occasions (arrows), separated by 120 min, over a 6-h period. To prevent an overload of the figure by statistical symbols, only significant differences between females and males are indicated (+, $p < 0.05$).

Table 2
Concentration of NA, DA, DOPAC, 5-HT and 5-HIAA in various brain structures of male (MALE) and female (FEMALE) rats subjected to MDMA or NACL injections in Experiment 2

| | NA | DA | DOPAC | 5-HT | 5-HIAA |
|---|---|---|---|---|---|
| *Prefrontal cortex* | | | | | |
| MALE RATS | | | | | |
| NACL | 70.7±9.9 | 152.3±58.2 | 38.8±7.6 | 196.0±23.6 | 105.6±9.5 |
| MDMA 2MG | 75.2±10.3 | 88.4±23.2 | 43.7±7.4 | 259.2±21.6 | 120.7±9.1 |
| MDMA 5MG | 75.6±8.9 | 102.6±39.5 | 32.7±6.3 | 236.7±12.7 | 112.9±8.5 |
| MDMA 10MG | 72.7±20.5 | 167.0±85.6 | 63.6±29.3 | 139.9±29.7 | 104.2±14.0 |
| FEMALE RATS | | | | | |
| NACL | 87.0±6.1 | 206.5±67.9 | 54.0±11.1 | 240.0±19.7 | 152.4±8.2 |
| MDMA 2MG | 87.4±7.1 | 187.8±47.9 | 48.2±7.9 | 296.2±25.4 | 145.6±8.9 |
| MDMA 5MG | 84.3±6.9 | 369.6±33.1 | 109.3±12.5 | 252.5±20.1 | 164.4±9.3 |
| MDMA 10MG | 87.4±3.2 | 203.2±46.2 | 51.7±6.4 | 252.9±19.4 | 128.3±4.8 |
| *Frontal cortex* | | | | | |
| MALE RATS | | | | | |
| NACL | 64.8±6.8 | 94.9±25.0 | 27.6±2.9 | 104.5±11.0 | 86.7±8.2 |
| MDMA 2MG | 73.0±5.7 | 112.2±15.6 | 31.0±1.6 | 136.5±12.2 | 101.7±7.0 |
| MDMA 5MG | 72.3±3.8 | 99.4±14.5 | 28.8±2.3 | 138.1±5.1 | 101.1±9.5 |
| MDMA 10MG | 75.2±12.5 | 73.7±20.3 | 26.5±2.2 | 102.9±19.6 | 87.0±10.6 |
| FEMALE RATS | | | | | |
| NACL | 113.1±4.6 | 114.6±23.5 | 29.8±4.6 | 173.1±13.9 | 134.1±10.6 |
| MDMA 2MG | 111.8±7.4 | 168.0±26.9 | 34.3±4.6 | 171.7±8.6 | 124.2±4.9 |
| MDMA 5MG | 118.9±2.7 | 217.6±40.4 | 40.3±4.2 | 182.5±12.8 | 134.2±10.2 |
| MDMA 10MG | 132.7±5.5 | 160.4±27.2 | 38.1±3.1 | 193.2±21.6 | 129.3±9.0 |
| *Temporal cortex* | | | | | |
| MALE RATS | | | | | |
| NACL | 100.2±3.3 | 22.6±5.1 | 15.7±2.8 | 96.3±12.9 | 81.6±12.5 |
| MDMA 2MG | 105.7±8.5 | 35.5±12.1 | 21.0±5.4 | 117.7±11.6 | 102.6±16.1 |
| MDMA 5MG | 110.1±8.1 | 24.4±3.0 | 12.5±0.8 | 134.8±3.7 | 88.0±5.0 |
| MDMA 10MG | 86.3±10.2 | 18.2±6.4 | 16.4±2.6 | 77.1±18.4 | 63.4±11.5 |
| FEMALE RATS | | | | | |
| NACL | 94.2±1.9 | 18.8±2.4 | 28.6±2.2 | 129.5±4.2 | 98.2±3.5 |
| MDMA 2MG | 94.5±3.9 | 27.6±3.8 | 31.3±1.4 | 142.4±6.1 | 93.6±1.8 |
| MDMA 5MG | 101.8±4.3 | 26.4±5.0 | 31.7±1.8 | 139.5±11.4 | 95.6±7.9 |
| MDMA 10MG | 106.1±6.2 | 31.2±6.1 | 31.6±2.1 | 121.8±7.7 | 82.6±5.5 |
| *Occipital cortex* | | | | | |
| MALE RATS | | | | | |
| NACL | 45.6±6.7 | 9.0±1.5 | 10.8±1.6 | 139.2±39.4 | 81.1±12.8 |
| MDMA 2MG | 49.9±6.8 | 12.0±2.5 | 9.1±1.3 | 115.6±13.8 | 80.2±3.9 |
| MDMA 5MG | 84.7±7.6 | 11.6±2.0 | 5.9±0.9 | 175.7±23.3 | 94.0±9.7 |
| MDMA 10MG | 86.5±7.5 | 8.48±1.7 | 5.4±0.6 | 156.3±21.4 | 86.1±11.5 |
| FEMALE RATS | | | | | |
| NACL | 77.0±5.6 | 9.7±2.5 | 5.8±0.9 | 164.5±18.7 | 109.7±5.9 |
| MDMA 2MG | 76.1±5.9 | 10.9±1.6 | 4.6±0.7 | 155.0±11.5 | 88.6±3.4 |
| MDMA 5MG | 84.7±7.6 | 11.6±2.0 | 5.9±0.9 | 175.7±23.3 | 94.0±9.7 |
| MDMA 10MG | 86.5±7.5 | 8.5±1.7 | 5.4±0.6 | 156.3±21.4 | 86.1±11.5 |
| *Dorsal hippocampus* | | | | | |
| MALE RATS | | | | | |
| NACL | 97.6±4.7 | 44.7±8.1 | 40.6±4.0 | 151.7±15.3 | 181.4±22.0 |
| MDMA 2MG | 110.1±8.3 | 41.5±5.8 | 44.6±4.4 | 203.4±19.5 | 222.6±16.0 |
| MDMA 5MG | 95.3±5.3 | 46.9±4.9 | 38.1±3.4 | 178.5±8.7 | 197.2±12.4 |
| MDMA 10MG | 90.0±7.4 | 24.6±3.6 | 37.1±2.8 | 116.5±22.0 | 145.4±21.8 |
| FEMALE RATS | | | | | |
| NACL | 63.6±2.8 | 33.1±6.4 | 22.8±6.1 | 186.3±9.1 | 229.9±9.8 |
| MDMA 2MG | 69.8±5.4 | 37.6±4.4 | 25.7±3.7 | 203.5±13.6 | 230.8±14.7 |
| MDMA 5MG | 72.7±7.9 | 37.2±6.4 | 23.2±4.5 | 199.3±23.5 | 233.0±26.5 |
| MDMA 10MG | 58.4±8.0 | 38.6±15.7 | 26.3±7.8 | 142.8±26.2 | 174.1±32.6 |

Table 2 (*continued*)

| | NA | DA | DOPAC | 5-HT | 5-HIAA |
|---|---|---|---|---|---|
| *Ventral hippocampus* | | | | | |
| MALE RATS | | | | | |
| NACL | 117.8±16.2 | 25.1±11.2 | 21.3±3.3 | 224.1±27.2 | 239.6±29.3 |
| MDMA 2MG | 147.5±24.7 | 19.9±4.0 | 20.7±3.9 | 298.9±35.3 | 308.3±29.2 |
| MDMA 5MG | 133.7±9.2 | 17.8±1.4 | 17.8±2.9 | 296.1±13.2 | 283.3±13.5 |
| MDMA 10MG | 115.3±15.7 | 43.4±31.5 | 45.6±30.0 | 154.3±35.2 | 169.3±33.0 |
| FEMALE RATS | | | | | |
| NACL | 157.7±15.8 | 17.4±1.3 | 10.9±0.9 | 333.4±26.4 | 344.4±27.9 |
| MDMA 2MG | 154.6±9.5 | 16.2±1.2 | 10.4±0.7 | 330.6±12.2 | 306.7±17.3 |
| MDMA 5MG | 175.2±17.3 | 25.1±5.3 | 10.9±1.1 | 305.0±25.6 | 304.6±30.8 |
| MDMA 10MG | 175.6±10.7 | 15.4±4.7 | 9.4±2.0 | 294.6±25.9 | 300.1±28.2 |

was due to overall temperatures that were significantly higher (i) at the delay of +440 min as compared to all previous ones ($p<0.05$ at least), (ii) at the delay of 320 min as compared to −45 min, 40 min and 80 min ($p<0.001$), (iii) at the delays of +200 min and +280 min as compared to all previous ones ($p<0.05$), (iv) at the delay of +160 min as compared to all previous ones ($p<0.001$), and finally (v) at the delay of +80 min as compared to both previous ones ($p<0.001$). Interestingly, while the Sex × Dose ($F$ 3/46=2.3, $p=0.09$) and the Sex × Injection ($F$ 7/322=1.4) interactions were not significant, the Dose × Injection ($F$ 21/322=14.9, $p<0.001$) as well as the Sex × Dose × Injection ($F$ 21/322=2.4, $p<0.001$) interactions were significant. The significant Dose × Injection interaction was mainly due to temperatures that were significantly higher in MDMA 5MG rats as compared to MDMA 2MG or NACL rats, but only following the second injection ($p<0.05$ at least), as well as in MDMA 10MG as compared to MDMA 5MG, MDMA 2MG and NACL rats, mainly after the second injection ($p<0.001$). At the delay of 80 min, the temperature in MDMA 10MG rats was also significantly higher than in MDMA 2MG and NACL rats ($p<0.001$). Finally, the Sex × Dose × Injection can be interpreted as reflecting a larger temperature increase in MALE MDMA 10MG as compared to FEMALE MDMA 10 MG rats at delays of +200 min, +280 min, +320 min and +440 min ($p<0.01$, at least). At the doses of 2 or 5 mg/kg, there was no significant difference between males and females, whatever post-injection delay was considered.

In summary: at the dose of 2 mg/kg, there was no significant increase in temperature, regardless of sex or treatment. At 5 mg/kg, there was a significant increase in temperature after the second injection, observed in both sexes, but with no sex difference. At 80 min after the second injection, male rats given 10 mg/kg MDMA showed a larger temperature increase than their female counterparts, a difference that persisted until and after the 3rd injection.

### 3.2.2. Monoamine concentrations

All data are shown in Table 2. For each brain region in which monoamine levels were measured and each monoamine or metabolite, data were analyzed by Sex × Dose (0, 2, 5, 10 mg/kg) factorial design. There was a significant overall Sex effect on the concentration of noradrenaline, DA, DOPAC, 5-HIAA and 5-HT in almost all structures ($F$ 1/43> 4.0, $p<0.05$); exceptions were noradrenaline in the temporal cortex ($F$ 1/43<1.0), dopamine in the occipital and temporal cortex, as well as in the dorsal hippocampus ($F$ 1/43<1.0), DOPAC in the ventral hippocampus ($F$ 1/43<1.0), 5-HIAA in the temporal cortex ($F$ 1/43=2.0), and 5-HT in the dorsal hippocampus ($F$ 1/43=2.5). When significant, the Sex effect was due to overall values that were significantly higher in FEMALES as compared to MALES ($p<0.05$ at least), except for noradrenaline in the dorsal hippocampus, DA in the ventral hippocampus, and DOPAC in the occipital cortex and the dorsal hippocampus, where the values were significantly higher in MALES ($p<0.05$ at least). A significant overall Dose effect was found for 5-HT in the prefrontal and temporal cortex, as well as in the dorsal and ventral hippocampus ($F$ 1/43>4.0, $p<0.05$). In the prefrontal cortex this Dose effect was due to concentrations that were significantly larger in rats given the dose of 2 mg/kg as compared to the NACL-treated ones. In the hippocampus, rats given 10 mg/kg had 5-HT concentrations that were significantly reduced as compared to those given lower doses of MDMA or Saline ($p<0.05$). In none of the regions was there a significant Sex × Drug interaction for any amine or metabolite.

### 3.3. Discussion of Experiment 2

Experiment 1 focused on the locomotor effects of MDMA. Based on a physiological parameter sensitive to MDMA, namely body temperature, this second experiment further supports our first conclusion: in case of repeated treatment, pubescent male rats appear more sensitive to a high dose of MDMA than their female age-matched counterparts. Actually, the hyperthermia induced by the highest dose of the drug started to be more pronounced in males after the second of three cumulative MDMA injections, and this difference was clear-cut after the third injection. In this second experiment, we also observed that the serotonin depletion induced by the drug was less pronounced than in our first one. This difference is not necessarily surprising, as we used a lower ambient temperature in order to perform this second experiment with

increased survival chances in males. Previous studies have shown that hyperthermia played an important role in serotonin depletion by MDMA (e.g., O'Loinsigh et al., 2001). In addition, the extent of MDMA-induced hyperthermia was shown to depend upon ambient temperature (Malberg and Seiden, 1998). Thus, the reduced serotonin levels found in our second experiment were probably a direct consequence of the lower ambient temperature. Interestingly, we also found concentrations of some of the monoamines or metabolites to be different between males and females, most of them being higher in females. Surprisingly, the literature on a possible sexual dimorphism in catecholamine and indolamine levels in the cortex or the hippocampus is rather sparse. These sex-dependent differences can nevertheless be linked to a few previous reports showing a sexual dimorphism in, for instance, dopaminergic or other monoaminergic functions (e.g., Crowley et al., 1978; Reisert et al., 1989; Restani et al., 1990; see also De Vries, 1990).

## 4. General discussion

In humans, including adolescents, it is not uncommon for those attending a "rave" or other social function involving the use of MDMA to take multiple doses amounting to 300 or more mg in one session; regular users typically take 2 or 3 tablets, whereas those more experienced consume much more (e.g., Parrott, 2005). In the present study, we administered MDMA 3 times at 2 h intervals. The dosing equivalence may be estimated according to an interspecies scaling principle (Mordanti and Chappell, 1989), although interspecies equivalences for MDMA dosing or other aspects of MDMA treatment are difficult to defend (De la Torre and Farré, 2004). Keeping the limitation of allometric scaling across models in mind (De la Torre and Farré, 2004), pubescent rats were used as an animal model of MDMA consumption in young adolescents, and repeated injections to model dosing patterns, as observed in regular ecstasy users (e.g., Parrott, 2005; Winstock et al., 2001). This protocol is not intended to replicate exactly consumption situations as they occur in humans. Rather it is intended to capture some of the aspects of repeated dosing at short intervals and assess the effects and associated possible risks. De la Torre and Farré (2004) caution us about the limitations of such models, notably because of inconsistencies among animal species, including humans, in terms of metabolic dispositions or pathways, pharmacokinetics and other factors. Despite these drawbacks, the model enabled us to address the question of MDMA effects in pubescent rats of both sexes and to show that under some conditions, males exhibit higher sensitivity to this drug than do females.

Neurochemically, MDMA causes an acute and rapid release of serotonin and dopamine (Green et al., 2003), which is accompanied, among other manifestations (Green et al., 2003) by hyperthermia and hyperlocomotion. In rats, the hyperthermic response most probably involves an action of dopamine on $D_1$ (Mechan et al., 2002), perhaps also on $D_2$ (Dafters and Biello, 2003) receptors, while the hyperlocomotion may have both dopaminergic and serotonergic components, involving at least $D_1$, $D_2$ and 5-$HT_2$ receptors (Kehne et al., 1996; see Green et al., 2003 for a recent and comprehensive review). Hyperthermia is thought to play a crucial, though not exclusive, role in MDMA lethality (e.g., Green et al., 2003; Schifano, 2004). In the first experiment, the body temperature of the rats could not be recorded concomitantly with the activity measures. Therefore, the hyperpyretic effects of MDMA were recorded in a second experiment in a separate cohort of rats.

The main contribution of our present experiment is confirmation that male pubescent rats appear more sensitive to repeated treatments of MDMA than females. Indeed, with repeated treatment, males exhibit higher activity, body temperature, and even lethality rate.

Concerning the hyperactivity, it is known that estrogens may enhance the locomotor response to MDMA (e.g., Zhou et al., 2003). However, because males showed significantly greater responses than females after the second injection, this hypothesis may need re-evaluation. As to the sex-based differences in death rates, and this speculation might also account for the differences in locomotion, it is possible that they simply reflected a difference in the metabolism of MDMA. To our knowledge, data on sex differences in MDMA metabolism in rats are not available, except in Sprague–Dawley rats. After treatment with MDMA, the brain concentration of 3,4-methylenedioxyamphetamine (MDA), one of the major metabolites of MDMA, was lower in females than in males, while the brain and plasma concentrations of MDMA remained comparable between both sexes. This suggests a slower metabolism in females, presumably because of a lower N-demethylation activity (Chu et al., 1996; see also Colado et al., 1995). If such differences also existed in Long–Evans rats, it would be possible to link part of our data to them. The larger concentration of MDA in the male brain could actually account for both a higher locomotor activity and a stronger thermal response because (at least a metabolite of) MDA has also been shown to stimulate locomotion (Easton et al., 2003) and induce hyperthermia (Colado et al., 1995). Alternatively, the reduced MDA in brain but not plasma of the females may indicate a decreased distribution to the brain of the parent drug. In the present study, sex-dependent differences were found at 10 mg/kg. According to a recent article by De la Torre and Farré (2004), this dose in rats is close to the saturation of hepatic MDMA metabolism, and this metabolism might be different between females and males: if slower in females and if one or more MDMA metabolites account for the behavioral and physiological effects of the drug, it can be that males show more intense responses under a regimen of repeated administration.

Concerning lethality, it is more difficult to establish such a relationship with MDMA metabolites, because the exact mechanism underlying its lethality is presently unknown (e.g., Schifano, 2004); it is almost certain that hyperthermia is a major contributor, so research could be directed at the neurochemical processes involved. A direct action of MDA on $D_1$ and $D_2$ receptors can probably be excluded on the basis of the low affinity of this metabolite for both dopamine receptors (Battaglia et al., 1988).

Another possibility based on a metabolic factor to account for sex-dependent differences might consider other hormonal pathways, e.g., the hypothalamic-pituitary-adrenal (HPA) axis. For instance, MDMA may interact with glucocorticoid release (e.g., Yau et al., 1997), the effects of MDMA can be accentuated by glucocorticoid treatment or stress (e.g., Johnson et al., 2004), and a sexual dimorphism has been described as concerns functions of the HPA axis (e.g., Atkinson and Waddell, 1997). Finally, males were slightly heavier than females (about 23 g in Experiment 1 and about 18 g in Experiment 2), and although we injected a constant dose and not a constant amount of MDMA, it cannot be excluded that factors related to body size and weight (e.g., amount of plasma) also contributed to account for the observed difference (e.g., bioavailability of the drug might have been greater in males). At our present state of knowledge and in the absence of experimental data, it is nevertheless difficult to speculate further on this issue.

As an alternative to a metabolic explanation, or perhaps even a complementary mechanism, it is also tempting to speculate on sex-specific effects related to differences in dopamine receptors. Indeed, the difference between females and males might have partly stemmed from differences in MDMA-induced changes in dopaminergic neurotransmission involving $D_1$, and perhaps also $D_2$ receptors. Interestingly, Andersen et al. (1997) reported sex-related differences in both $D_1$ and $D_2$ dopamine receptors in the rat striatum, particularly at the time of puberty onset (i.e., PND 40). Between PND 25 and PND 40, the production of striatal $D_1$ receptors increased by 65% in males (vs. 35% in females), and that of $D_2$ receptors increased by 144% (vs. 31% in females). The peak values (and also the largest male vs. female differences) were reached at PND 40. Afterwards, the density of receptors decreased to nearly comparable levels in both sexes. Interestingly, the authors also provided data showing that these raises in receptor productions occurred independently from pubertal gonadal hormones (Andersen et al., 2002). As our MDMA injections were made in rats at age PND 39, it is possible that the higher sensitivity of pubescent males, whether in terms of locomotor activity, hyperthermia or death rate, may be linked to this transient overproduction of $D_1$ and $D_2$ receptors. This issue must be addressed in a future study, perhaps using different ages.

Taken as a whole, our present results clearly indicate a sexual dimorphism in the sensitivity to MDMA in pubescent rats: females initially, then males appear to be more sensitive to cumulative treatment with MDMA. Whatever the reason accounting for this difference may be, it is noteworthy that the observation after the second injection is at some variance with the dimorphism described for other psychostimulant effects, like amphetamine or cocaine, to which females respond more markedly than males. We do not know by now how to account for this striking difference, which is nevertheless supported by convergent observations: locomotion, body temperature and lethality were larger in males than in females after the second of three cumulative MDMA injections. Sex-dependent metabolic, pharmacokinetic and/or pharmacodynamic factors might account for this difference, but there is a clear need for further experimentation to identify which of them is/are involved.

Whether this dimorphism may be linked to the reported sex ratios of fatal cases in young human beings remains to be addressed more thoroughly. In addition, it must once again be emphasized that extrapolating from animal studies to humans is all the more difficult because several confounding factors may have contributed to the fatal issue in humans, including co-administration of MDMA with other drugs (users being often polydrug abusers) and variability in individual sensitivity, experience, purity of the drug, sociocultural and environmental factors (e.g., De la Torre and Farré, 2004; Green et al., 2003; Parrott, 2005; Schifano, 2004).

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