

An analysis of spontaneous behavior following acute MDMA treatment in male and female rats

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Abstract

3,4-methylenedioxymethamphetamine (MDMA, ecstasy) is a widely abused drug that impairs behavioral, emotional and cognitive functions in humans and animals. The aim of this study was to evaluate MDMA effects on the spontaneous behavioral repertoire of rats with a focus on the gender differences.

MDMA was given subcutaneously in a single dose of 2.5, 5 and 10 mg/kg and the spontaneous behavior of male and female rats was studied using the open field test. Behavioral patterns (locomotion, rearing, floor-sniffing, air-sniffing, grooming, immobility and stereotypy) were registered in two sessions – 30 and 60 min following MDMA administration; each session lasting 5 min.

We found that MDMA totally disrupted the structure and timing of spontaneous behavioral patterns in both genders; no evident differences were measured between either of the sessions. MDMA irrespective of the dosage produced hyperlocomotion, excessive floor-sniffing and almost absolute suppression of grooming and immobility. A biphasic effect of MDMA was found in rearing. Gender differences were present namely in rearing and sniffing stereotypy. This study also confirms that behavioral experiments should focus on more behavioral elements than only on e.g. locomotion and that the observer-based approach still gives the most reliable results.

INTRODUCTION

Ecstasy (3,4-methylenedioxymethamphetamine, MDMA) is one of the most frequently abused designer drugs amongst youngsters (Landry, 2002; Tossman *et al.*, 2001). With respect to its psychoactive effects in humans, it belongs to the group of

drugs called entactogens (Nichols and Oberlender, 1990). In rats, MDMA is known to produce a typical behavioral syndrome characterized by hyperthermia, hyperactivity, piloerection, salivation, mydriasis, ataxia and abnormal limb movements,

low body posture, splayed hind limbs, and reciprocal forepaw treading (Green *et al.*, 1995). In many aspects these somatic and behavioral phenomena resemble pure serotonin syndrome (Marona-Lewicka and Nichols, 1994; Pranzatelli *et al.*, 1987).

It has been reported that MDMA exhibited a predominantly excitatory effect on neuronal activity that was positively correlated with the locomotor activation. MDMA increased locomotor activity and decreased rearing in rats, produced thigmotaxis in the open field arena and decreased exploratory holepokes behavior (Callaway *et al.*, 1990; Paulus and Geyer, 1992; O'Loinsigh *et al.*, 2001; McCreary *et al.*, 1999; Gold *et al.*, 1989; Palenicek *et al.*, 2005). Even though there are many studies in humans describing behavioral and psychoactive effects of MDMA, little is known about gender differences in responsiveness to the drug. Only a few studies in humans as well as in animals refer to some gender differences. In most of these studies females tend to be more sensitive to the effect of MDMA (Liechti *et al.*, 2001; Reneman *et al.*, 2001; McCann *et al.*, 1994; Milani *et al.*, 2004; Verheyden *et al.*, 2002; Rodgers *et al.*, 2003; Koenig *et al.*, 2005; Miller and O'Callaghan, 1995; Chu *et al.*, 1996; Colado *et al.*, 1995; Palenicek *et al.*, 2005; Bubenikova *et al.*, 2005).

However, the exact pattern of behavior and the MDMA administration was not described and also the behavioral difference between males and females are restricted to the description of motility. Up until now there has been no detailed comparative study examining the behavioral repertoire of male and female rats after acute MDMA. This study was therefore designed to evaluate MDMA effects on the spontaneous behavioral repertoire of rats. In order to acquire a precise insight in behavioral patterns, not only the frequency but also the duration of particular patterns was evaluated using the open field test. To evaluate if and to what an extent changes in behavioral repertoire can persist, the behavioral repertoire was recorded 30 and 60 min following MDMA administration. Since MDMA treated animals have been shown to exhibit stereotypical behaviors (Antoniou and Kafetzopoulos, 1991; O'Loinsigh *et al.*, 2001), we have also additionally analyzed atypical and stereotypical patterns.

MATERIALS AND METHODS

Animals

Male (180–220 g, N=48) and female (150–170 g, N=40) Wistar rats (Biotest s.r.o., Konárovice, Czech Republic) were housed in a 12 h light/dark regime with a temperature ranging from 22 to 24 °C and free access to standard diet and water. Rats were given an acclimatization period of 10 days prior to the start of the experiment. Experiments were approved by the Expert Committee for Protection of Experimental Animals of the 3rd Faculty of Medicine and were performed in accordance with the Animal Protection Act of the Czech Republic.

Drug Treatment

3,4-methylenedioxymethamphetamine hydrochloride (99% purity by GC-MS; Pharmaceutical Faculty of Charles University, Hradec Králové, Czech Republic) was dissolved in saline (0.9% NaCl w/v) and administered subcutaneously (s.c.) as a single dose of 2.5, 5.0, and 10.0 mg/kg in a volume 2 ml/1 kg of body weight. 0.9% NaCl was used as a placebo treatment.

Behavioral Procedure

The behavioral testing was performed under room lighting from 8 a.m. to 1 p.m. (during the light phase of the cycle). Animals were housed separately in cages immediately following the drug administration. Each animal was tested in the rectangular arena (68×51×35 cm, made from transparent plexiglass), 30 min (session 1) and repeatedly 60 min (session 2) after the drug administration. The observation session lasted 5 min. The arena was placed in a sound-proof equally lit room. The floor surface of the arena was optically divided to 4×3 identical squares. The behavior of rats was recorded by typing pre-set keys on the keyboard of a computer. Activities software registered the number of pressings and the time (in seconds) between each pressing.

The following behavioral patterns (total number and time spent) were distinguished: (a) Locomotion activity was expressed by the total number of crossed squares, (b) Rearing was recorded when an animal raised both forelegs from the ground and either rested them on the cage wall or not, (c) Floor sniffing means that an animal investigated the arena floor usually with movements of vibrissae and the head tilted upwards, often accompanied by licking the floor, (d) Air sniffing was expressed as a quadrupedal stance with scanning movements of the head oriented toward the environment, (e) Stereotyped sniffing was expressed as an increase in the time of continuous uninterrupted sniffing, (f) Grooming was recorded when an animal exhibited vibrational movements of the forelegs, washing of the forelegs and the head, cleaning of the hind legs, body, tail and genitals, (g) Immobility means that an animal stands or reposes (lying, freezing).

Concomitantly animals were registered on a videotape and the record was stored for subsequent analysis of additional behavioral parameters. A trained observer, blind to the treatment conditions, viewed videotapes of experimental sessions and scored the occurrence of the different atypical behavioral actions, which were identified during observation: fast one-way locomotion along the wall without any or with short stopping in the corners; creeping and slow locomotion with the abdomen closely by the floor; foot shuffling – an animal performs short excursions (several forward steps) from the home base; fast locomotion with the head permanently directed to the floor; fast locomotion with the head permanently up; locomotion along the longer and the back wall with a change of direction in corners; body rotation on the spot; rapid and repetitive head movements from one side to the other;

grooming intention in the course of locomotion (means that an animal rubs the forelegs shortly against the nose).

Statistics

All data were analyzed with a two-way ANOVA being performed for gender and treatment effects. In the case of significance it was followed by a post hoc Tukey's test. Repeated behavioral measurements (i.e. session 1 versus session 2) were analyzed using paired T-tests within particular treatment groups and independently on the gender. Differences were considered as significant for $p < 0.05$. All analyses were performed using SigmaStat for Windows v 3.0.

RESULTS

Total number of all registered patterns

In both sessions MDMA produced a significant increase in both males and females (Figure 1). There was a significant difference between the controls and all MDMA doses ($p < 0.001$ in all cases). When first versus second session was compared, a significant decrease was observed in the male control group ($p = 0.003$) and in both genders within the 2.5 mg/kg MDMA dose ($p = 0.026$ for males, $p = 0.015$ for females) in the second session. No gender differences were found.

Total number of individual patterns

Crossing (Figure 2): In both sessions MDMA produced a significant increase in both genders. There was a significant difference between the controls and all MDMA doses ($p = 0.001$ in all cases). Compared to the first session, a significant decrease was observed in male controls ($p = 0.005$) and in both genders within the 2.5 mg/kg dose ($p = 0.017$ for males, $p = 0.001$ for females) in the second session; furthermore, a significant increase of crossing was found in the 10 mg/kg MDMA treated males ($p = 0.025$). No gender differences were found.

Rearing (Figure 3): In the first session MDMA produced a significant increase in rearing in females given the 2.5 mg/kg dose ($p = 0.017$); this increase was also significant when compared to the male group ($p < 0.001$). In contrast, a significant reduction was obvious within the 10 mg/kg dose in males ($p = 0.02$) and females ($p = 0.006$). In the second session, when compared to the controls, a significant higher number of rearing was found in all MDMA 2.5 mg/kg treated animals ($p = 0.006$ for males and $p < 0.001$ for females). An explicit reduction of rearing was found in males given the highest MDMA dose. Though not significant when compared to the controls, it revealed significantly different compared to females ($p < 0.001$). As compared to the first session, a significant lower level of rearing in the controls of both genders was registered during the second session ($p = 0.013$ for males, $p = 0.003$ for females) and in the 10 mg/kg male group ($p = 0.031$). On the contrary, there was an increase in the 2.5 mg/kg male group ($p = 0.017$), both 5 mg/kg groups ($p = 0.014$ for males and $p = 0.023$ for females) and in 10 mg/kg female group ($p = 0.006$).

Total time spent in individual patterns

Rearing (Figure 4): In the first session, compared to the controls MDMA produced a significant decrease in rearing in both genders for doses 5 mg/kg ($p = 0.02$ for males, $p = 0.003$ for females) and 10 mg/kg ($p < 0.001$ for both genders). In contrast, for the 2.5 mg/kg dose the females spent significantly more time in rearing than males ($p = 0.009$). In the second session only the females given the 2.5 mg/kg dose spent more time in rearing than the controls ($p = 0.021$). In males the 10 mg/kg dose produced a significant reduction in the time devoted to rearing as compared to the controls ($p < 0.001$). Furthermore, there was a significant difference between the 2.5 and 10 mg/kg MDMA treatment in both genders ($p < 0.001$ for males, $p = 0.01$ for females). At the 10 mg/kg dose males spent significantly less time in rearing than females ($p < 0.001$).

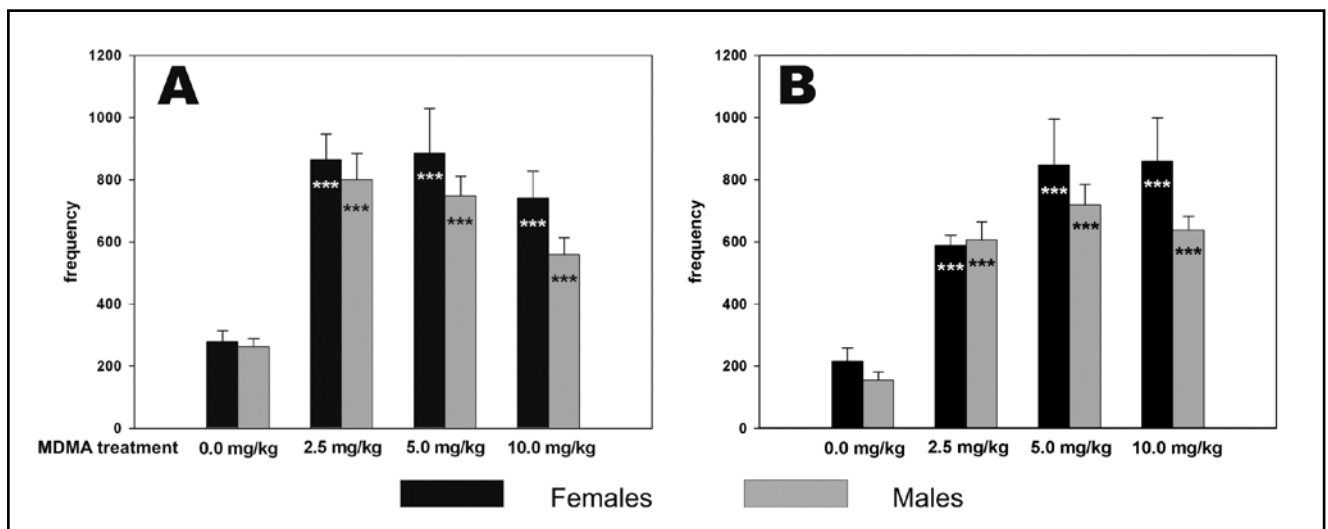


Figure 1. Effect of MDMA (2.5, 5 and 10 mg/kg s.c.) on the frequency of all behavioral patterns at 30 min (A) and 60 min (B) after the treatment in rats measured for 5 minutes (***) $p < 0.001$ for the difference vs. control groups, a two-way ANOVA with post-hoc Tukey t-tests).

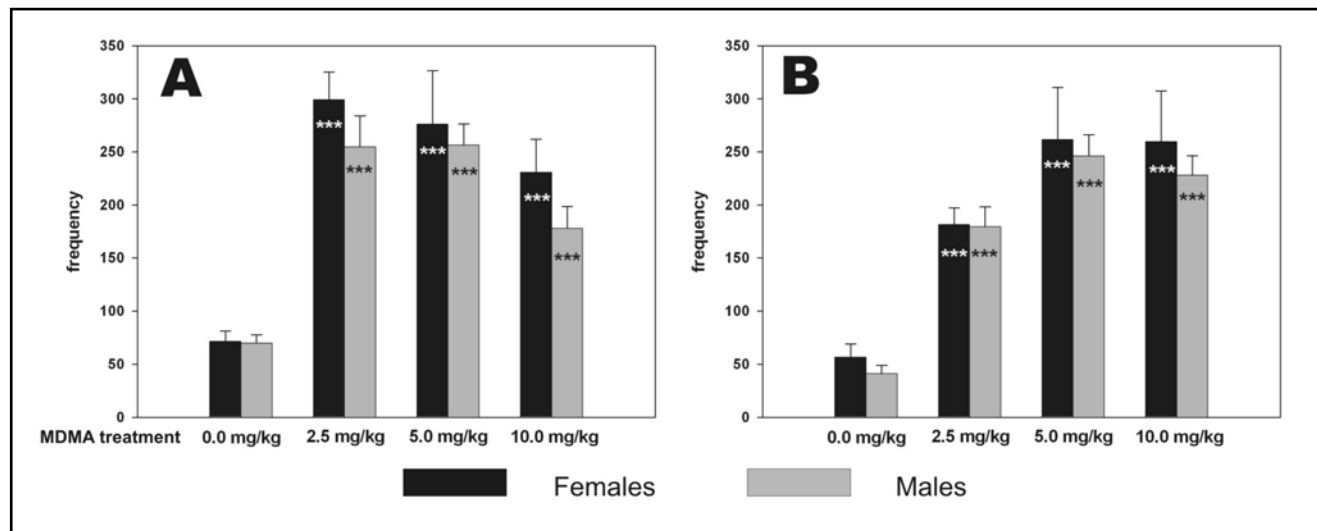


Figure 2. Effect of MDMA (2.5, 5 and 10 mg/kg s.c.) on the frequency of crossings at 30 min (A) and 60 min (B) after the treatment in rats measured for 5 minutes (** $p < 0.001$ for the difference vs. control groups, a two-way ANOVA with post-hoc Tukey t-tests).

Compared to the first session, there was a significant decrease during the second session in the time in female controls ($p = 0.004$) and in the 10 mg/kg MDMA treated males ($p = 0.029$); on the contrary, a significant increase was found in the 10 mg/kg female group ($p = 0.01$).

Floor sniffing (Figure 4): In both sessions MDMA produced a significant increase floor sniffing in both genders without a difference between sessions. In the first session, as compared to the controls, significantly more time was spent in the 2.5 mg/kg group ($p < 0.001$ for males, $p = 0.008$ for females), 5 mg/kg group ($p < 0.001$ for both genders), and 10 mg/kg group ($p < 0.001$ for males, $p = 0.03$ for females). In the second session, there was a significant difference between the controls and the 2.5 mg/kg group ($p = 0.006$ for males, $p = 0.03$ for females), 5 mg/kg group ($p = 0.006$ for males, $p < 0.001$ for females), and 10 mg/kg group ($p < 0.001$ for both genders). Moreover, for MDMA

5 mg/kg females spent significantly more time in floor sniffing than males ($p = 0.039$).

Air sniffing (Figure 4): In the first session, females given the 2.5 mg/kg dose spent less time in air sniffing than those treated with the 10 mg/kg dose ($p = 0.003$); no difference was found between those given MDMA 2.5 mg/kg and the controls. In the second session, a significant increase was found only in males given 10 mg/kg dose as compared to the controls ($p = 0.023$). A gender difference was revealed for the 5 mg/kg dose where males spent more time in air sniffing than females ($p = 0.04$). No difference was found between both sessions.

Grooming (Figure 4): A significant reduction in MDMA treated animals of both genders was found in both sessions. There was a significant difference between the controls and all MDMA doses ($p < 0.001$ in all cases). During the second session females given the 2.5 mg/kg

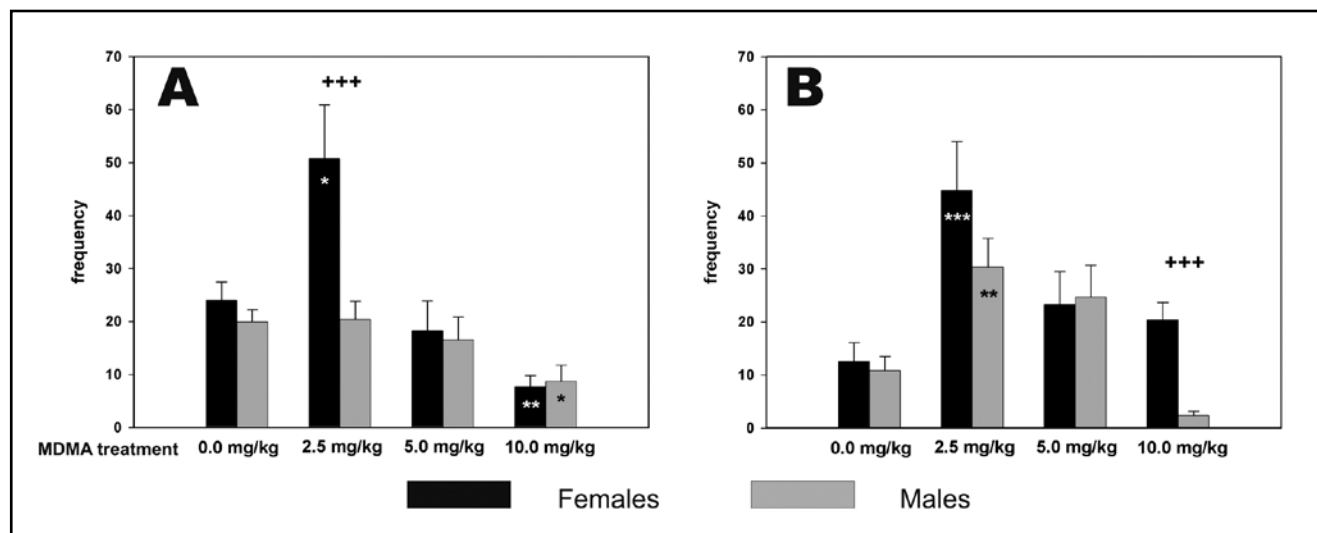


Figure 3. Effects of MDMA (2.5, 5 and 10 mg/kg s.c.) on the frequency of rearing at 30 min (A) and 60 min (B) after the treatment in rats measured for 5 minutes (** $p < 0.001$, * $p < 0.01$, * $p < 0.05$ for the difference vs. control groups; +++ $p < 0.001$ for the difference between males and females, a two-way ANOVA with post-hoc Tukey t-test).

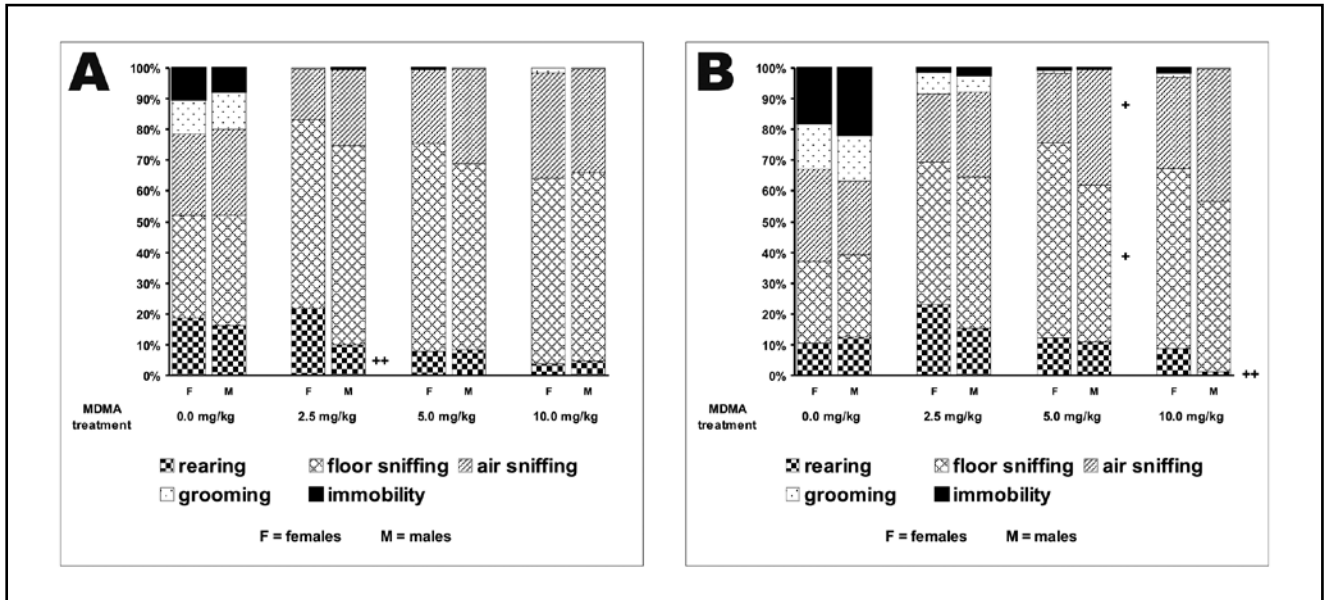


Figure 4. Effects of MDMA (2.5, 5 and 10 mg/kg s.c.) on time spent in each activity at 30 min (A) and 60 min (B) after the treatment in rats measured for 5 minutes, expressed as a percentage (+ $p<0.05$, ++ $p<0.01$ for the difference between males and females, a two-way ANOVA with post-hoc Tukey t-test).

dose spent more time in grooming than in the first session ($p=0.002$). No gender differences were found.

Immobility (Figure 4): As compared to the controls, MDMA irrespective of the dose produced almost absolute loss of immobility in both genders ($p<0.001$ in all cases) in both sessions. No difference between sessions was found.

Atypical and stereotypical behavior

Atypical behavior: In addition to the above mentioned patterns, MDMA treated animals exhibited some atypical behavior not observable in the controls (*see materials and methods*). In both sessions a dose-dependent effect

of MDMA treatment was observed, significantly marked at 5 mg/kg MDMA ($p=0.003$ for males and $p=0.005$ for females), and 10 mg/kg MDMA ($p<0.001$ for both genders) during the first session and in 10 mg/kg MDMA ($p<0.05$ for both genders) during the second session. A gender difference was almost present for MDMA 2.5 mg/kg during the first session ($p=0.059$), no differences between sessions were found.

Sniffing stereotypy (Figure 5): Analysis of sniffing stereotypy revealed significant dose and gender differences in both sessions. During the first session sniffing stereotypy was observed at MDMA 5 mg/kg females ($p=0.012$) and 10 mg/kg in both genders ($p<0.001$). How-

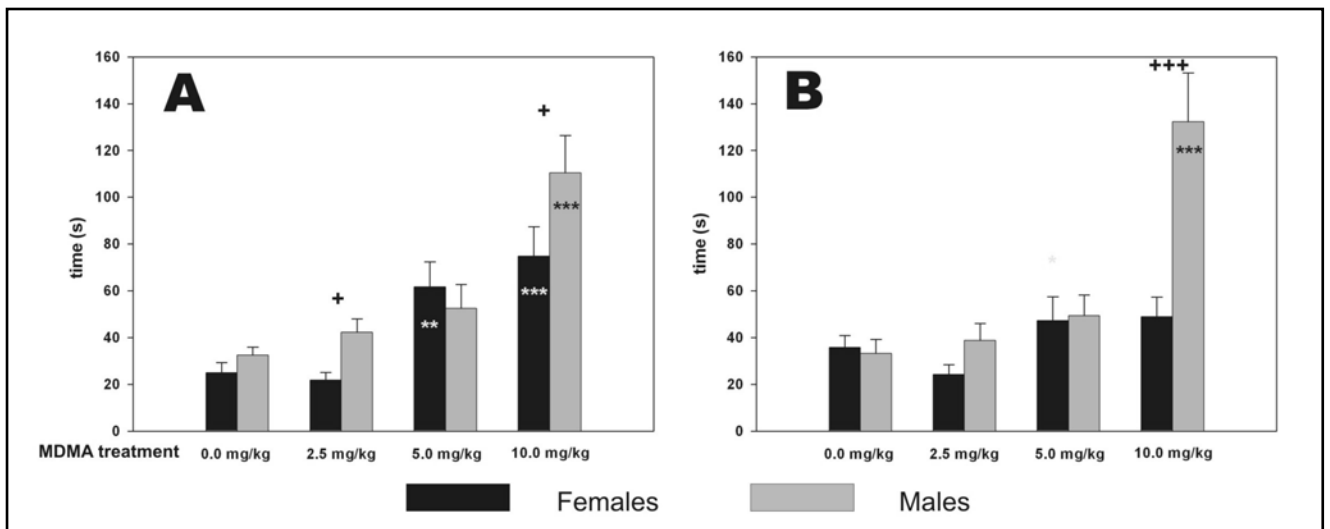


Figure 5. Effects of MDMA (2.5, 5 and 10 mg/kg s.c.) on the time spent in continuous uninterrupted (stereotyped) sniffing at 30 min (A) and 60 min (B) post MDMA treatment (*** $p<0.001$, ** $p<0.01$ for the difference vs. control groups; + $p<0.05$, +++ $p<0.001$ for the difference between males and females, a two-way ANOVA with post-hoc Tukey t-test).

ever, during the second session only the male 10 mg/kg group exhibited significantly more stereotypes than the controls ($p < 0.001$). Male rats exhibited more stereotypy in both sessions than females: the differences were found within 2.5 mg/kg and 10 mg/kg ($p < 0.05$) during the first session and within 10 mg/kg ($p < 0.001$) during the second session. There was a significant reduction in stereotypy in females given 10 mg/kg between sessions ($p = 0.02$).

DISCUSSION

The results show that MDMA affects behavioral structure and timing of all behavioral patterns in rats. MDMA has general stimulatory effects, indicated by an increase of all registered patterns and nearly absolute suppression of immobility in our setting. Next, we observed gender differences in some behavioral patterns after MDMA administration.

The most remarkable result is an increase in locomotion, which is consistently present in other studies (Bankson and Cunningham, 2002; Palenicek *et al.*, 2005; McCreary *et al.*, 1999; Ball *et al.*, 2003; Bubar *et al.*, 2004; Scarce-Levie *et al.*, 1999) and there was a slight but not significant increase in total number of all activities and crossing in female rats.

However, the most prominent gender differences were found in the rearing and sniffing behavior. In general, we observed an increase of rearing with the lowest MDMA dose (2.5 mg/kg) and a decrease with the two higher doses (5 and 10 mg/kg). On the contrary to males, there was more pronounced rearing behavior both in the frequency and the time spent in females given 2.5 mg/kg MDMA; this effect was evident during the first session with a trend in the second session. Males exhibited almost no rearing at 10 mg/kg MDMA during the second session. The observed changes in rearing could also reflect a measure of anxiety by means of the exploration. In our previous study high doses of MDMA had anxiolytic effects in the elevated plus maze (Palenicek *et al.*, 2005). Others have shown increased anxiety with low doses of MDMA and decreased anxiety with high doses of MDMA (Ho *et al.*, 2004). Thus increased rearing behavior could be a measure of increased anxiety leading to increased exploratory behavior and a decrease as the opposite.

A very constant effect of all three dosages was the strong potentiation of sniffing behavior, especially floor sniffing; changes in air sniffing were less consistent. After the 5 mg/kg MDMA administration the proportion in time spent in floor and air sniffing differed between genders during the second session: total time spent in air sniffing was decreased and floor sniffing was increased in females. On the other hand, male rats exhibited more sniffing stereotypy in both sessions. This was apparent in 2.5 mg/kg MDMA in the first session and 10 mg/kg MDMA for both sessions. Interestingly females during the second session did not exhibit almost any uninterrupted periods of continuous sniffing. Although the total

duration of sniffing was nearly identical in both genders, it emerged that continuous sniffing was more often interrupted by short rearing in females than in males. This is presumably the reason why sniffing stereotypy was not so pronounced in females.

Fast locomotion with the head permanently down (directed to the floor) is a typical and the most frequent indicator of MDMA treated rats. In the controls the total time spent in all sniffing behavior (irrespective of genders) was approximately 60% of the observation period, while in MDMA treated animals it reached 90% and more. A more detailed analysis of this behavior showed that running across the walls accompanied by continuous sniffing behavior was frequently present in longer periods compared to the control animals, indicating that such behavior can be termed as a stereotypy (Ball *et al.*, 2003; Gold *et al.*, 1989; Paulus and Geyer, 1992). Others also report different stereotypy like lateral head waving and forepaw treading (Fone *et al.*, 2002; Spanos and Yamamoto, 1989).

Behavioral changes induced by MDMA persisted at least for one hour. Several other studies have shown that the effect on locomotion and rearing persists as long as two hours post MDMA administration (Ball *et al.*, 2003; Gold *et al.*, 1989; McCreary *et al.*, 1999). While in controls some indicators of habituation were observed (crossing and rearing), in MDMA treated animals only a certain decrease in the magnitude of effects at a dose of 2.5 mg/kg MDMA was observed.

MDMA also displaced the grooming behavior; treated animals did not exhibit almost any such behavior. In the present study, control animals, irrespective of the gender, spent approximately 12 % of the observation period in grooming behavior. On the contrary, MDMA treated animals exhibited almost no grooming, they displayed only a single foreleg stroke over the face or nose by one or both paws. Since an animal did not continue in grooming, it is possible to term the action as a displacement activity. It seems conceivable that because of the hyperstimulation (locomotion and floor sniffing behavior) induced by MDMA the elicitation of grooming and immobility, a behavior closely associated with habituation, was eliminated.

The data coming from animal studies with MDMA regarding gender differences are very limited. A study of Koenig *et al.* describes increased sensitivity of male pubescent Long-Evans rats to MDMA induced hyperthermic effects and locomotor stimulation (Koenig *et al.*, 2005). No gender differences in behavioral parameters in Sprague-Dawley rats and rhesus monkeys after MDMA treatment have been observed (Slikker, Jr. *et al.*, 1989). In mice, increased lethality of males after MDMA exposure was reported (Miller and O'Callaghan, 1995). Some gender differences in the metabolism of MDMA in Sprague-Dawley rats were also observed (Chu *et al.*, 1996). It also has to be mentioned that Dark Agouti female rats are used as a model of the CYP2D6 poor metabolisers of MDMA (Colado *et al.*, 1995). Finally in the test of pre-

pulse inhibition of acoustic startle reaction (PPI), male rats, diestrous and metestrous female rats exerted higher deficits in PPI than proestrous and estrous female rats (Bubenikova *et al.*, 2005). More recently we described an increased sensitivity of female rats to locomotor stimulant effects of MDMA, however no gender differences in the elevated plus-maze (Palenicek *et al.*, 2005). Observed gender differences in locomotor stimulant effects in our previous study (the activity cage and in the computerized video-tracking open field) (Palenicek *et al.*, 2005) are likely to be due to better sensitivity of these methods to measure locomotion than manual scoring of crossing used in the current study. However manual scoring has evidently better detection of other behavioral patterns. On the other hand we have proved the same biphasic effect on rearing in female rats as in our previous study (Palenicek *et al.*, 2005).

Different sensitivity of serotonergic, noradrenergic and dopaminergic systems within genders to released monoamines, gender differences in the metabolism of MDMA as well as the role of sex hormones contribute to observed gender differences. These aspects have been discussed in our previous studies (Bubenikova *et al.*, 2005; Palenicek *et al.*, 2005); however explanation of observed differences on neurochemical basis would need further experiments with selective ligands for particular receptors.

In conclusion, we provide a detailed insight into the changes of spontaneous behavior of rats treated with MDMA. The behavioral changes are not restricted to locomotion, as is presented in many experimental studies. Changes of behavioral patterns are prominent also in the stereotyped behavior accompanied by floor sniffing, while markers of habituation – grooming and immobility, almost disappear. Gender differences were present namely in rearing and sniffing stereotypy. Finally, this study confirms that behavioral experiments should focus on more behavioral elements than only on e.g. locomotion and that the observer-based approach still gives the most reliable results.

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