Relation of sex and estrous phase to deficits in prepulse inhibition of the startle response induced by ecstasy (MDMA)

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Sensorimotor gating is the ability of a weak sensory event to inhibit the motor response to an intense stimulus. Drugs that act as serotonin releasers, such as MDMA (3,4-methylenedioxymethamphetamine), impair sensorimotor gating, which is measured as a prepulse inhibition (PPI) of the acoustic startle response. The first objective of the present study was to compare the effect of different doses of MDMA on PPI and the acoustic startle response (ASR) in male and female Wistar rats. The second objective was to examine the effect of MDMA on PPI across the estrous cycle in female rats. MDMA was administered in doses of 2.5, 5 and 10 mg/kg s.c. 15 min before the start of the experiment. The controls received saline in equivalent volumes. MDMA dose-dependently decreased PPI in both the male and female rats and produced higher levels of ASR in the male rats compared to the females. In addition, we found that female rats in the diestrous and metestrous phases are more sensitive to MDMA and showed higher deficits in PPI than female rats in the proestrous and estrous phases. Our result showed that female rats in the proestrous and estrous phases were less sensitive to the disruption of PPI by MDMA. *Behavioural Pharmacology* 16:127–130 © 2005 Lippincott Williams & Wilkins.

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Introduction

Sensorimotor gating is the ability of a weak sensory event to inhibit the motor response of an intense stimulus; it is thought to be governed by central inhibitory mechanisms (Swerdlow *et al.*, 2001). Sensorimotor gating can be measured by prepulse inhibition (PPI) of the acoustic startle response (ASR), reflecting the ability of the brain to filter sensory information (Koch, 1999).

Habituation and PPI can be disrupted by manipulation of different neurotransmitter systems, such as the dopaminergic system in the striatum, the GABAergic and serotonergic systems in the ventral pallidum or the cholinergic system in the hippocampus (Swerdlow *et al.*, 1992; Sipes and Geyer, 1997; Vollenweider *et al.*, 1999; Geyer *et al.*, 2001). It was found that agonists of the serotonin receptors 5-HT_{1A}, 5-HT_{2A} and 5-HT_{1D} reduced PPI in rats (Sipes and Geyer, 1994, 1995) and that drugs that act as serotonin releasers also impair PPI and habituation (Vollenweider *et al.*, 1999).

3,4-Methylenedioxymethamphetamine (MDMA), known as Ecstasy, is widely used as a recreational drug (Burgess *et al.*, 2000). It binds to monoamine transporters, exhibiting the highest affinity for the serotonin 5-HT transporter and about tenfold lower affinities for the norepinephrine and dopamine transporters (Green *et al.*, 2003). However, there is evidence that the primary target of MDMA is inhibition of the presynaptic serotonin transporter and, consequently, an enhanced release of serotonin. This activates both presynaptic serotonin receptors 5-$HT_{1A/1B}$ and postsynaptic serotonin receptors, such as 5-HT_{1A} and 5-$HT_{2A/2C}$ (Bankson and Cunningham, 2001). The effect of MDMA is also associated with an increased level of extracellular dopamine, which is related to its activation of 5-$HT_{2A/2C}$ receptors and to its effect on the dopamine transporter (Yamamoto *et al.*, 1995; Simantov, 2004).

It was found that ovarian steroids in different parts of the rat brain (McEwen, 2002) affect the levels of dopamine and serotonin. Based on this study, the possibility of differences in the effect of MDMA on sensorimotor gating between males and females and across the estrous cycle is supported. There is growing evidence, based on animal and clinical studies, that females are more susceptible to the effects of drugs of abuse (Carroll *et al.*, 2004), but very little is known about possible sex differences in the effects of MDMA. To date, no published studies have examined the effect of MDMA on sensorimotor gating across the estrous cycle in female rats. Therefore, we compared the effect of MDMA on PPI in female rats according to their estrous phase.

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Methods

Subjects

All experiments were carried out on adult male Wistar rats (SPF animals; Hannover breed Konárovice, Czech Republic) weighing 200–250 g and adult female rats weighing 160–180 g. In each experimental group, 8–13 animals were used. For comparative analysis, data from the females in the metestrous (total 30 rats) and diestrous phases (total 15 rats) were grouped, as well as from the females in the proestrous (total 19 rats) and estrous phases (total 20 rats). All experiments respected the Guidelines of the European Union Council (86/609/EU) and followed the instructions of the National Committee for the Care and Use of Laboratory Animals.

Study design

All rats were initially tested in a short session (a 5 min acclimatization period plus five single pulses) to determine the drug-free baseline startle magnitude, 2 days before the experiment. On the experimental day, MDMA or saline was administrated 15 min before the start of the testing session. Vaginal smears were taken from the female rats after the testing session. Each estrous phase was determined according to the study by Marcondes *et al.* (2002).

Startle and PPI apparatus and experimental schedule

All testing was performed in the startle chamber (SR-LAB, San Diego Instruments, California, USA). A high-frequency loudspeaker inside the chamber produced both a background noise of 62 dB and the acoustic stimulus, 120 dB.

The experimental design was modified from that of Schulz *et al.* (2001). After the acclimatization period (5 min) the test began with five initial startle stimuli (120 dB) followed by four different trial types, presented in a pseudo-random order: (1) single pulse: 120 db broadband burst, 20 ms duration; (2) prepulse: 13 dB, 20 ms duration above the background noise, presented 100 ms before the onset of the pulse alone; (3) prepulse alone: 13 dB, 20 ms duration above the background noise; (4) no stimulus. Five presentations of each trial type were given with an interstimulus interval of about 30 s. The PPI was expressed as a percentage of PPI [100 – (mean response for the prepulse–pulse trials/startle response for the single-pulse trials) × 100]. The four single pulse trials at the beginning of the test session were not included in the calculation of the PPI values. Animals that had an average value lower than 10 were excluded from the calculation of the PPI and were marked as non-responders. The number of animals removed did not differ significantly among all treatment groups.

Drugs

MDMA was dissolved in saline and administered in doses of 2.5 mg/kg, 5 mg/kg or 10 mg/kg subcutaneously (s.c.) in a volume of 2 ml/kg. Controls received saline injection of an equivalent volume.

Statistical analysis

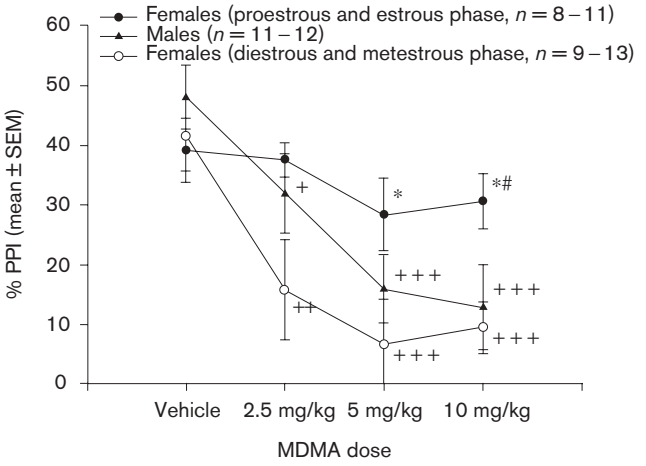
Data from both parts of the experiment (ASR and PPI) were collected and evaluated statistically by two-way analysis of variance (ANOVA), with MDMA treatment (control, 2.5, 5 and 10 mg/kg) as one factor and sex (males, females and females according to estrous phases) as the second factor. The two-way ANOVA was followed by subsequent comparisons between the treatment groups using a Student–Newman–Keuls *post-hoc* test; α level was $P < 0.05$ (SigmaStat 3.0, SPSS Icn. Chicago, USA).

Results

When female rats were divided according to their estrous phase (the proestrous and estrous females in one group, and the metestrous and diestrous females in the second group), a two-way ANOVA, with one factor represented by treatment and the second factor, sex (male, female proestrous + estrous, and female metestrous + diestrous), showed a significant effect of the treatment [$F(3,128) = 12.273$, $P < 0.001$] and sex [$F(2,128) = 6.177$, $P < 0.01$] on the PPI. The subsequent Student–Newman–Keuls test did not show any significant difference between females in the proestrous + estrous phases, females in the metestrous + diestrous phases and on the PPI without MDMA treatment. The MDMA treatment had a strong effect on males and females in the metestrous + diestrous phase. The lowest dose of MDMA (2.5 mg/kg) decreased PPI in both males ($P < 0.05$) and females in the metestrous + diestrous phase ($P < 0.01$) compared to the control animals in the congruent phase (Fig. 1). The decrease in PPI was more pronounced after the dose of 5 mg/kg MDMA in males ($P < 0.001$) and in females in the metestrous + diestrous phase ($P < 0.001$), respectively. The biggest effect of MDMA was found at the 10 mg/kg dose of MDMA in males ($P < 0.001$) and in females in the metestrous + diestrous phase ($P < 0.001$).

Subsequent analysis showed significantly higher PPI in the females in the proestrous + estrous phase compared to that of the females in the metestrous + diestrous phase after the 5 mg/kg dose of MDMA ($P < 0.05$). After the 10 mg/kg dose of MDMA we found significantly higher PPI in the females in the proestrous + estrous phase compared to PPI in both the males ($P < 0.05$) and females in the metestrous + diestrous phase ($P < 0.05$) (Fig. 1). A two-way ANOVA with the first factor, treatment, and the second factor, sex (male, female proestrous + estrous, and female metestrous + diestrous), did not show any significant difference in ASR between the groups of rats (data not shown).

Fig. 1



The effect of 3,4-methylenedioxymethamphetamine (MDMA; Ecstasy) (2.5 mg/kg, 5 mg/kg and 10 mg/kg s.c.) on prepulse inhibition (PPI) during the estrous cycle in female rats and in male rats. Data are expressed as mean of %PPI ± SEM, n=8–13 animals for each data plot. +, $P<0.05$; + +, $P<0.01$; + + +, $P<0.001$ for the difference relative to the control group of animals in the congruent phase; *, $P<0.05$ for the difference relative to female rats in the metestrous and diestrous phase; and #, $P<0.05$ for the difference relative to the male rats (a two-way ANOVA with *post-hoc* Student–Newman–Keuls test).

Discussion

In the present study, we evaluated changes in PPI in males and in females according to their estrous phase. We found that female rats in the diestrous and metestrous phases were more sensitive to MDMA and exerted higher deficits in PPI than female rats in the proestrous and estrous phases. Male rats showed similar deficits in PPI as the female rats in the diestrous and metestrous phase after administration of 5 mg/kg or 10 mg/kg MDMA. On the other hand, we did not observe any changes in PPI during the estrous cycle in the control female rats. Our data show that males and females in the diestrous and metestrous phases yield similar PPI responses after administration of high doses of MDMA. In addition, the level of ASR was not changed in males and was not affected by the estrous phase in the controls and treated female rats.

It seems that the pharmacological sensitivity to MDMA is dependent on changes of gonadal hormones during the estrous cycle in female rats. Estrogen and progesterone, the main gonadal hormones in female rats, reach a maximum toward the end of proestrous, and estrogen has a second maximum in the middle of the estrous phase. Our experiments were done between 08.00 and 13.00 hours, when the level of estrogen is the highest in both the proestrous and estrous phases in female rats (Spornitz *et al.*, 1999). Our result shows that estrogen and progesterone prevent the disruption of PPI in female rats by MDMA. During the metestrous and diestrous phases in female rats, when levels of hormones are at a minimum, the MDMA produced disruption of PPI similar to that produced in male rats.

MDMA belongs to the group of substrate-type releasers that produce a robust increase of the extracellular serotonin level by a process that is independent of cell firing (Rothman and Baumann, 2002). Serotonin released from the terminals by MDMA can activate 14 distinct serotonin receptors.

Ovarian steroids are associated with changes in the expression of serotonin receptors, transporter and metabolism, which result in a higher level of serotonin available for release (Ramirez and Carrer, 1982; Gundlah *et al.*, 1998; McQueen *et al.*, 1999; McEwen, 2002). Thus, female rats in the proestrous and estrous phases would be expected to exert a greater response to MDMA. However, our results show that female rats in the estrous and proestrous phases are less sensitive to MDMA than rats in the metestrous and diestrous phases.

One possible explanation of our findings is that ovarian steroid hormones prevent the disruption of PPI in female rats. It has been shown previously that estrogen and progesterone prevent disruption of PPI by systemic administration of a serotonin 5-HT_{1A} agonist (Gogos and Van den Buuse, 2004). In accord with these findings, the disruptive effect of MDMA on PPI could be triggered by activation of the serotonin 5-HT_{1A} receptor. During the estrous and proestrous phases, the level of ovarian steroids desensitizes the serotonergic 5-HT_{1A} receptor (Carrasco *et al.*, 2004) and then the effect of MDMA on PPI is exerted more intensely in the metestrous and diestrous phases of female rats.

This is the first evidence that the estrous phase influences the deficits in PPI induced by acute administration of MDMA, and more experiments are needed to fully establish an influence of ovarian steroids on MDMA and other substrate-type releaser-induced behavior.

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