Progress in Neuro-Psychopharmacology & Biological Psychiatry 34 (2010) 588–596

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Sex differences in the effects of N,N-diethyllysergamide (LSD) on behavioural activity and prepulse inhibition

Tomáš Páleníček *, Zdeněk Hliňák, Věra Bubeníková-Valešová, Tomáš Novák, Jiří Horáček

Prague Psychiatric Center, Ústavní 91, 181 03, Prague 8, Czech Republic

ARTICLE INFO

Article history:
Received 18 December 2009
Received in revised form 7 February 2010
Accepted 7 February 2010
Available online 13 February 2010

Keywords:
N,N-Diethyllysergamide (LSD)
Locomotion
Open field
Prepulse inhibition
Rats
Sex differences

ABSTRACT

The aim of this study was to describe sex differences in the behavioural effects of N,N-diethyllysergamide (LSD) (locomotor activity and other behavioural repertoire in the open field) and its effects on sensorimotor gating in rats (prepulse inhibition (PPI) of the acoustic startle reaction). Three groups of animals were analysed: males, oestral and pro-oestral phase females (EP females), and metoestral and dioestral phase females (MD females). LSD (5, 50 and 200 μg/kg subcutaneously) attenuated locomotor activity and normal behavioural repertoire, and induced flat body posture, wet dog shakes and disrupted PPI. The most prominent behavioural findings of LSD were for LSD 200 μg/kg which suppressed almost all behavioural activity. LSD had mainly inhibitory locomotor effects in males and MD females, yet in EP female rats LSD increased locomotion during the second half of testing period. The main sex differences were observed in locomotor and exploratory behaviour. Both EP and MD females were less sensitive to hypolocomotor effects of LSD and had less pronounced thigmotaxis than males. Further EP females had increased rearing after LSD 5 μg/kg. On the contrary although LSD disrupted PPI in males and MD female rats, EP females were protected from this disruptive effect. Thus, EP females seem to have a lower sensitivity to LSD behavioural actions.

1. Introduction

The hallucinogenic drug N,N-diethyllysergamide (LSD) is one of the most potent hallucinogenic compounds, the main action of which is suggested to be via agonism at serotonin 5-$HT_{2A/C}$ and 5-HT_{1A} receptors (Egan et al., 1998; Marek and Aghajanian, 1996, 1998; Nichols et al., 2002). Agonism at serotonin 5-HT_{5A} and dopamine D_1 and D_2 receptors also appears to contribute to its effects (Grailhe et al., 1999; Krebs-Thomson and Geyer, 1996; Marona-Lewicka et al., 2005; Nichols et al., 2002; Seeman et al., 2009; Watts et al., 1995).

LSD has been reported to alter locomotor and exploratory behaviour, sensorimotor gating, and anxiety in rodents (Adams and Geyer, 1985a; Cunha and Masur, 1978; Geyer et al., 1979; Kabes et al., 1972; Kabes and Fink, 1971; Ouagazzal et al., 2001). However, most of the studies have been carried out on male rats and mice. Only a few studies, some of which are over 30 years old, have been performed on female rats (Horowski, 1983; Horowski and Wachtel, 1979; Selye, 1971). Even though many drugs of abuse including serotonergic drugs like 3,4-methylenedioxymethamphetamine (MDMA) have been reported to show specific sex differences in their behavioural action as well as toxicity (Allott and Redman, 2007; Bubenikova et al., 2005; Carroll et al., 2004; Palenicek et al., 2005, 2007b; Roth et al., 2004), there is only limited evidence of sex differences in the effects of LSD in animals (Meehan and Schechter, 1998); others found no sex differences (Kabes and Fink, 1971). In humans, the most frequently studied psychedelic compounds in the past were LSD, psilocybin and mescaline (Monroe et al., 1957; Roubicek and Srnec, 1955; Schwarz et al., 1956; Wolbach, Jr. et al., 1962). However, we did not find any reference about sex differences in these studies. More recent studies with tryptamine and phenylethylamine psychedelics do not have a sufficient number of male/female volunteers to compare sex differences (Gouzoulis-Mayfrank et al., 2005; Hasler et al., 2004; Hermle et al., 1992; Vollenweider et al., 1998, 2007). Another psychedelic compound, ketamine, is studied intensively in humans yet it has a different mechanism of action (Abel et al., 2003; Fu et al., 2005; Gouzoulis-Mayfrank et al., 2005; Krystal et al., 1994). Again, there are no data on sex differences even though such expectations may be justified on the basis of animal studies (Lees et al., 2004). On the contrary, there is evidence suggesting higher sensitivity in women to MDMA induced psychological and toxicological effects (Liechti et al., 2001; McCann et al., 1994; Reneman et al., 2001).

Further evidence refers to sex differences in serotonin 5-$HT_{2A/C}$ (Birzniece et al., 2002; Cyr et al., 2000; Sumner et al., 2007; Zhou et al., 2002) and 5-HT_{1A} receptor density and functionality (Blanchard et al., 1992; Frankfurt et al., 1994; Landry and Di, 2003; Le Saux and Di Paolo, 2005) which might specifically cause a divergence between the

Abbreviations: 5-MeO-DMT, 5-Methoxy-N,N-dimethyltryptamine; ANOVA, Analysis of variance; ASR, Acoustic startle reaction; DOS, Disk Operating System; E2, 17-β-Oestradiol; EP, Oestral and pro-oestral phases; GABA, γ-Aminobutyric acid; LSD, N,N-Diethyllysergamide; MD, Metoestral and dioestral phases; MDMA, 3,4-Methylenedioxymethamphetamine; PPI, Prepulse inhibition; TDT, Total distance travelled.

* Corresponding author. Prague Psychiatric Center, Ústavní 91, 181 03, Prague 8, Czech Republic. Tel.: +420 266003173; fax: +420 266003160.
E-mail address: palenicek@pcp.lf3.cuni.cz (T. Páleníček).

0278-5846/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pnpbp.2010.02.008

sexes in the behavioural action of LSD. Thus, the aim of this study was to evaluate the possible role of sex and female oestral cycling on the behavioural effects of LSD in rats. Since sex hormones influence functionality of the serotonergic system (Cosgrove et al., 2007; Fink et al., 1996), answering this question is relevant to both the clinical effects of LSD and its mechanism of action.

Locomotor activity and behaviour like sniffing, rearing, and grooming in the open field test describe the animal's ability to coordinate motor functions, and to explore and habituate in a novel environment. This test can also identify central stimulation versus depression. Most hallucinogenic drugs disorganize the typical behaviour of rats in the open field and influence typical locomotor patterns leading to a hypo- or hyperlocomotor effect or a biphasic mode of action (Geyer et al., 1979; Krebs-Thomson et al., 1998, 2006; Ouagazzal et al., 2001; Palenicek et al., 2005, 2008). Similarly, sensorimotor filtering and processing is typically disrupted by hallucinogenic compounds and this has been repeatedly demonstrated by a variety of studies with different psychedelic drugs including LSD using the test of prepulse inhibition (PPI) of acoustic startle reaction (ASR) (Bubenikova et al., 2005; Krebs-Thomson et al., 2006; Ouagazzal et al., 2001; Palenicek et al., 2008; Sipes and Geyer, 1997). The principle of this reaction is based on the fact that in mammals, and especially in rodents and humans, a preceding weak sensory event inhibits reaction to a subsequent intense stimulus which is measured as a magnitude of motor response. This reaction is thought to be governed by central inhibitory mechanisms, namely GABA-ergic neurotransmission, in the pontine reticular nucleus. Other brain structures (amygdala, frontal cortex, nucleus accumbens, and hippocampus) as well as neurotransmitters (glutamate, dopamine, and serotonin) modulate this reaction (Koch, 1999; Swerdlow et al., 2001).

In this study we report complex differences in the behavioural effects of LSD between male and female rats and between different stages of the oestrous cycle. We used three behavioural methods to analyse the influence of LSD on locomotion and its spatial characteristics, spontaneous behaviour in the open field and on sensorimotor gating in male and female rats. Since levels of 17-β-oestradiol (E2) and progesterone are highest during pro-oestrus and oestrus compared with metoestrus and dioestrus (Smith, 1994), female rats were divided into oestral and pro-oestral phase females (EP females), and metoestral and dioestral phase female (MD females) rats.

2. Materials and methods

2.1. Animals and procedure

Wistar male (200–250 g) and female (150–180 g) rats aged 8–9 weeks (Biotest s.r.o., Konárovice, Czech Republic) were housed in pairs in a 12 h light/dark regime with temperatures ranging from 22 to 24 °C and free access to a standard diet and water. The rats were given an acclimatization period of 7–10 days prior to the start of the experiment. During this period animals were weighed twice and handled four times during the cleaning of cages. Independent groups were used for each treatment (males, oestral and pro-oestral phase females (EP females), and metoestral and dioestral phase females (MD females)) and each animal was tested only once. Each group consisted of 8–12 animals (see in figures); the number of animals varied due to different numbers in oestral groups which were established after each experiment. To exclude the influence of odours, males and females were never tested on the same day. Preliminary vaginal smears were taken before each experiment in order to minimize the number of females used. These vaginal smears were taken at least 1 day prior to behavioural testing and helped estimate the cycle's phase on the testing day. On the testing day, vaginal smears were taken from the female rats immediately after the testing session. Each oestrous phase was determined according to the method used in the study by (Marcondes et al., 2002) and already used in our previous study (Bubenikova et al., 2005). A pro-oestrus smear consists of a predominance of nucleated epithelial cells, an oestrous smear primarily consists of anucleated cornified cells, a metoestrus smear consists of the same proportion of leukocytes, cornified, and nucleated epithelial cells and a dioestrus smear consists of a predominance of leukocytes. The experiments were approved by the Expert Committee for Protection of Experimental Animals of the 3rd Faculty of Medicine, Charles University in Prague, Czech Republic and were performed in accordance with the Animal Protection Act of the Czech Republic.

2.2. Drug treatment

N,N-diethyllysergamide (LSD, synthesized at the Pharmaceutical Faculty of Charles University, Hradec Králové, Czech Republic) in a form of freebase was dissolved in 20 µl of 96% ethanol and then adjusted to a required volume of 5 ml by saline (0.9% NaCl w/v) and administered subcutaneously (s.c.) as a single dose of 5, 50 and 200 µg/kg in a volume 2 ml/1 kg of body weight. A saline solution with 20 µl of 96% ethanol per 5 ml of saline was used as a placebo treatment.

2.3. Locomotor activity

Locomotor activity (total distance travelled, TDT) and its spatial characteristics (thigmotaxis) in a novel environment were registered and analysed by an automatic video tracking system for recording behavioural activity (EthoVision Color Pro v. 3.1.1, Noldus, Netherlands). A square black plastic box arena (68×68×30 cm) was situated in a sound-proof and evenly-lit room. Each rat was placed into the centre of the arena 15 min after drug administration (LSD 5, 50 and 200 µg/kg or vehicle) and locomotor activity was registered for 30 min. The EthoVision program was also used to calculate locomotor activity in 5-min time intervals. To evaluate the spatial characteristics of the movement in the open field (thigmotaxis) the arena was divided virtually by the EthoVision program into 5×5 identical square zones with 16 being located on the periphery and 9 in the centre. Initially, the total number of appearances of the animal in each zone (frequency; f) was calculated by the program. The thigmotaxis (i) was calculated as $i = f_{\text{peripheral zones}}/f_{\text{all zones}}$. Thus, the thigmotaxis is a relative number from 0 to 1 and indicates the probability of appearance in any of the peripheral zones within the arena (Palenicek et al., 2005, 2008).

2.4. Behavioural repertoire in the open field test

The behavioural testing in a novel environment was performed under room lighting from 8 a.m. to 12 a.m. (during the light phase of the cycle). Animals were placed back in their home cages immediately following drug administration. Each animal was tested in the rectangular arena (68×51×35 cm, made from transparent Plexiglas), 15 min after the drug administration (LSD 5, 50 and 200 µg/kg or vehicle). The observation session lasted 5 min. The arena was placed in a sound-proof and evenly-lit room. The floor surface of the arena was optically divided into 4×3 identical squares. The behaviour of the rats was recorded by typing pre-set keys on the keyboard of a computer. The Activities program (DOS based utility created in our laboratory) registered the number of key strokes and the time (in seconds) between each key stroke.

The following behavioural patterns (total number and time spent) were distinguished: (a) sniffing was recorded when an animal investigated the arena with vibrissae movements (b) rearing was recorded when an animal raised both forelegs from the ground and either rested them on the cage wall or not, (c) 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, (d) immobility meant that an animal stood or reposed (lied down or froze), (e) flat body posture meant the animal was laying or walking with its abdomen nestled against the floor of the arena,

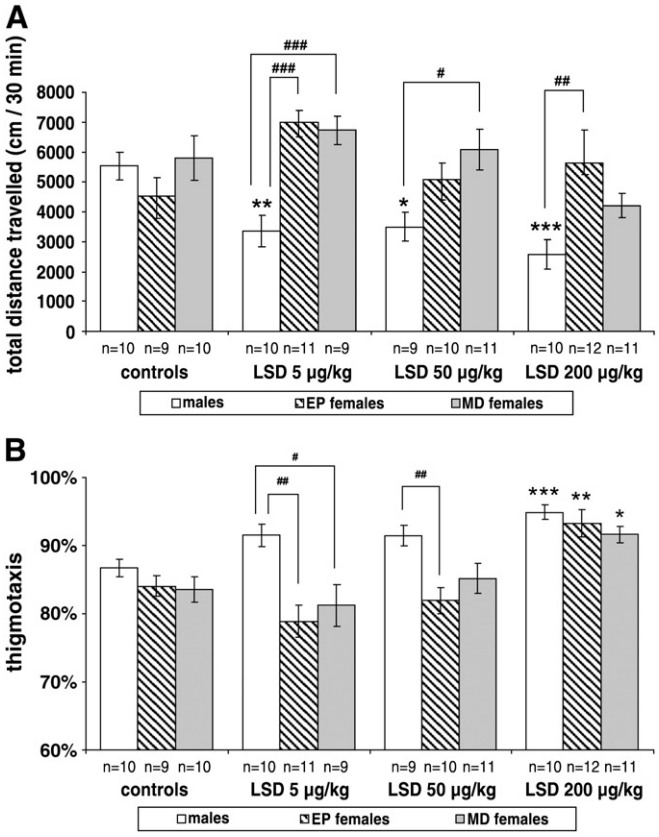

Fig. 1. (A) The effect of LSD on total distance travelled. LSD 5, 50 and 200 µg/kg decreased locomotion in male rats. Female rats exhibited more locomotor activity than male rats after LSD treatment. (B) The effect of LSD on thigmotaxis. LSD 200 µg/kg significantly increased thigmotaxis in all three groups. Males spent significantly more time on the periphery than EP and MD females after LSD 5 µg/kg and EP females after LSD 50 µg/kg. *, **, *** for $p<0.05$, 0.01 and 0.001 from respective control group, #, ##, ### for $p<0.05$, 0.01 and 0.001 between sex groups; *n* under each column indicates the number of animals per group.

(*f*) wet dog shakes were periods lasting 1–2 s when the animal shook the whole body or head.

2.5. Prepulse inhibition (PPI) of acoustic startle reaction (ASR)

All of the rats were habituated to the testing apparatus in a short session (a 5-min acclimatization period plus five single pulses) 2 days before the experiment. On the day of the experiment LSD (5, 50 and 200 µg/kg) or the vehicle was administered 15 min before the start of the testing session.

All testing was performed in two startle chambers (SR-LAB, San Diego Instruments, California, USA) which consist of a sound-proof, evenly-lit enclosure with a Plexiglas stabilimeter with 8.7 cm inner diameter. A piezoelectric accelerometer detected all peak and average amplitudes of the startle response. These were digitized and stored on a computer hard drive. A dynamic calibration system was used to ensure comparable stabilimeter sensitivity across the test chambers. Sound levels were measured using a RadioShack sound level meter. A high frequency loudspeaker mounted 24 cm above the Plexiglas cylinder inside the chamber produced both a background noise of 62 dB and all acoustic stimuli. The experimental design was adopted from previous studies (Bubenikova et al., 2005; Palenicek et al., 2008). 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–pulse: prepulse 13 dB above the background noise, 20 ms duration, presented 100 ms before the onset of the 120 dB pulse alone; (3) prepulse alone: 13 dB above the background noise, 20 ms duration; and (4) no stimulus. Five presentations of each trial type were given with a floating 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 with an average startle value lower than 10 were excluded from the calculation of the PPI and were marked as non-responders.

2.6. Statistics

The data were analysed using a two-way analysis of variance (ANOVA). Where a significant interaction in the between-subjects variables (group and treatment) was found, a subsequent one-way ANOVA was carried out followed by a post-hoc comparison using the Tukey–Kramer test. In the case of TDT in 5-min intervals, a three-way ANOVA with group and treatment as the between-subjects factors and time interval as a repeated measures factor was performed, and a Greenhouse–Geisser correction for nonsphericity was applied. This analysis was followed by a two-way ANOVA (group and treatment as the between-subjects factors) performed for TDT within a 30-min period and for each 5-min interval separately. A subsequent one-way ANOVA and a post-hoc comparison using the Tukey–Kramer test were used as in the previous analysis. The significance level was set at a p-value <0.05, although we considered a trend towards significance for p-values between 0.05 and 0.1. All analyses were performed using Statistica 7.0 software.

3. Results

3.1. Locomotor activity

3.1.1. Total distance travelled (TDT) (Fig. 1A, Table 1)

Overall analysis by two-way ANOVA revealed a significant effect for the treatment factor [$F(3,110)=3.65$, $p=0.015$], for the groups factor [$F(2,110)=12.60$, $p<0.001$] and for treatment × groups interaction [$F(6,110)=3.43$, $p=0.004$].

A significant treatment effect in males [$F(3,35)=7.32$, $p<0.001$] and almost reaching significance in EP females [$F(3,38)=2.51$, $p=0.073$] and MD females [$F(3,37)=2.7$, $p=0.06$] was found by one-way ANOVA. Compared with the control group, males given LSD 5, 50 and 200 µg/kg had significantly reduced TDT ($p<0.05$–$p<0.001$).

Table 1
Summary of significant changes observed after LSD treatments in male (M), EP females (EP) and MD females (MD). Significant changes ($p<0.05$ and lower) versus the control group are marked with − (decrease) or + (increase), n.s. (non significant). Gender differences with the direction of changes are typed underneath.

	LSD 5 µg/kg			LSD 50 µg/kg			LSD 200 µg/kg		
	M	EP	MD	M	EP	MD	M	EP	MD
TDT	−	n.s.	n.s.	−	n.s.	n.s.	−	n.s.	n.s.
		M<EP, MD			M<MD			M<EP	
Thigmotaxis	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	+	+	+
		M>EP, MD			M>EP				
Sniffing time	−	n.s.	n.s.	n.s.	n.s.	n.s.	−	−	−
Rearing time	n.s.	+	n.s.	n.s.	n.s.	n.s.	−	−	−
Grooming time	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	−	n.s.	−
Immobility time	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	+
Flat body posture time	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	+	+	+
Wet dog shakes frequency	n.s.	+	n.s.	+	+	+	n.s.	n.s.	n.s.
ASR	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
PPI	n.s.	n.s.	n.s.	−	n.s.	n.s.	n.s.	n.s.	−
					M>MD				

Subsequent comparisons of groups by one-way ANOVA showed no differences in TDT among the three control groups but there were differences in all LSD treatments: for LSD 5 [$F(2,27)=15.91$, $p<0.001$], for LSD 50 [$F(2,27)=3.44$, $p=0.047$] and for LSD 200 [$F(2,30)=6.05$, $p=0.006$]. The comparison with males showed significantly longer TDT in both EP and MD females treated with the lowest LSD dose ($p<0.001$), with the same effect being observed in MD females given the medium LSD dose ($p<0.05$) and in EP females with the highest LSD dose ($p<0.01$). Finally, no differences in TDT were found between EP and MD females irrespective of the LSD dose used.

3.1.2. Calculation of TDT in 5-min intervals (Fig. 2A–C, Table 2)

Three-way repeated measure ANOVA with group, treatment and time as the factors revealed a significant effect of groups [$F(2,110)=12.6$, $p<0.001$] and treatment [$F(3,110)=3.65$, $p=0.015$] and there was also a significant interaction between group and treatment [$F(6,110)=3.43$, $p=0.004$]. We also found a significant effect of time [$F(5,550)=515.07$, $p<0.001$] and a significant interaction between time × groups [$F(10,550)=2.76$, $p=0.008$], time × treatment [$F(15,550)=18.12$, $p<0.001$] and a trend of interaction between time × group × treatment [$F(30,550)=1.45$, $p=0.09$].

Subsequent two-way ANOVAs (Table 2) with group and treatment factors showed a significant effect of treatment at 0–5 min and 5–10 min and of groups in all periods. Except for the first period (0–5 min) we found a significant interaction for groups × treatment in all intervals.

The treatment effect was revealed by one-way ANOVA (Table 2) in males in the first four intervals. Post-hoc analysis showed that all LSD doses shortened TDT when compared to the male control group ($p<0.05$–$p<0.01$). In EP females the treatment effect was seen in all intervals except the second interval. Post-hoc analysis showed that compared to the EP female control group LSD 5 μg/kg and LSD 200 μg/kg increased TDT ($p<0.05$) in the second half of the measurement. In MD females the effects were seen during the first 10 min of measurement, where the post-hoc test showed that LSD 200 μg/kg significantly decreased TDT compared to the MD female control group ($p<0.001$).

Differences between male and MD and EP female groups (one-way ANOVA, Table 2) were seen in all intervals tested. Post-hoc analysis showed that males had significantly shorter TDT than EP and/or MD females ($p<0.05$–$p<0.001$) although there were no differences between EP and MD females in any period analysed.

To summarize these results, the males had significantly shorter TDT after LSD treatment compared to both groups of females. A closer look at the effects of LSD showed that in males and MD females, LSD decreased locomotion, mainly during the onset of its action (initial 15 min), while in EP females LSD increased locomotion in the second half of the testing period.

3.1.3. Thigmotaxis (Fig. 1B, Table 1)

Overall analysis by two-way ANOVA revealed a significant effect for the treatment factor [$F(3,110)=16.23$, $p<0.001$] and for the groups factor [$F(2,110)=14.28$, $p<0.001$] and a trend for treatment × groups interaction [$F(6,110)=2.15$, $p=0.054$].

The effects of treatment by one-way ANOVA were remarkable in all groups: males [$F(3,35)=6.03$, $p=0.002$], EP females [$F(3,38)=10.49$. $p<0.001$] and MD females [$F(3,37)=4.57$, $p=0.008$]. Post-hoc analysis showed that LSD 200 μg/kg significantly increased thigmotaxis in all groups compared to their respective control group ($p<0.05$–$p<0.001$).

In view of the fact that a trend in interaction in two-way ANOVA was present we attempted to conduct a one-way ANOVA between groups. We found no differences among the control animals and LSD 200 μg/kg, however, for LSD 5 μg/kg [$F(2,27)=8.24$, $p=0.002$] and for LSD 50 μg/kg [$F(2,27)=5.79$, $p=0.008$] males were significantly found more frequently in the periphery of the arena than EP and MD females as shown by post-hoc tests ($p<0.05$–$p<0.01$).

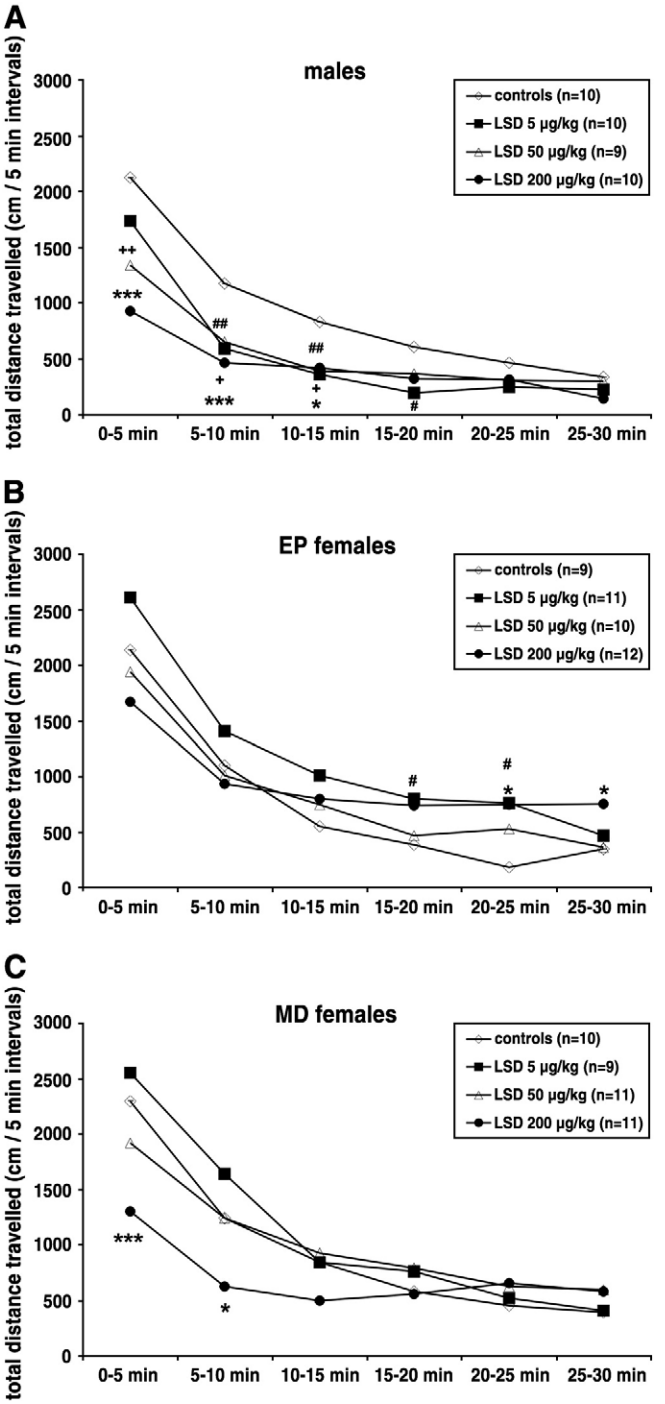

Fig. 2. The effect of LSD on total distance travelled in male rats (A), EP female rats (B) and MD female rats (C) divided in 5-min intervals. LSD 5, 50 and 200 μg/kg decreased locomotion during first four intervals in male (A) and MD female (C) rats, LSD 5 and 200 μg/kg increased locomotion during the last three intervals in EP female rats (B). # for $p<0.05$ and ## for $p<0.01$ indicates differences from the control group for LSD 5 μg/kg, + for $p<0.05$, ++ for $p<0.01$ for LSD 50 μg/kg and * for $p<0.05$ and *** for $p<0.001$ for LSD 200 μg/kg; n in parenthesis indicates the number of animals per group.

3.2. Behavioural repertoire in the open field test (Figs. 3 and 4, Tables 1 and 3)

Overall analysis by two-way ANOVA (Table 3) revealed a significant effect of treatment and of groups in most behavioural parameters. However treatment × groups interaction was not observed in any test, indicating no specific sex differences.

Table 2
Two-way ANOVA and one-way ANOVA results of locomotor activity in 5-min intervals. n.s. means non significant.

Time interval	0–5 min	5–10 min	10–15 min	15–20 min	20–25 min	25–30 min
Two-way ANOVA						
Treatment factor	$F(3,110)=27.02$, $p<0.001$	$F(3,110)=9.15$, $p<0.001$	n.s.	n.s.	n.s.	n.s.
Group factor	$F(2,110)=13.29$, $p<0.001$	$F(2,110)=11.94$, $p<0.001$	$F(2,110)=6.1$, $p=0.003$	$F(2,110)=7.00$, $p=0.001$	$F(2,110)=4.85$, $p=0.01$	$F(2,110)=5.67$, $p=0.005$
Treatment × group interaction	n.s.	$F(6,110)=3.25$, $p=0.006$	$F(6,110)=3.08$, $p=0.008$	$F(6,110)=3.19$, $p=0.006$	$F(6,110)=2.70$, $p=0.02$	$F(6,110)=2.28$, $p=0.04$
One-way ANOVA — treatment effect						
Males	$F(3,35)=15.47$, $p<0.001$	$F(3,35)=7.80$, $p<0.001$	$F(3,35)=5.54$, $p=0.003$	$F(3,35)=3.46$, $p=0.027$	n.s.	n.s.
EP females	$F(3,38)=5.06$, $p=0.005$	n.s.	n.s.	$F(3,38)=3.49$, $p=0.02$	$F(3,38)=3.76$, $p=0.018$	$F(3,38)=4.05$, $p=0.014$
MD females	$F(3,37)=15.53$, $p<0.001$	$F(3,37)=7.63$, $p<0.001$	n.s.	n.s.	n.s.	n.s.
One-way ANOVA — group effect						
Controls	Not tested	n.s.	n.s.	n.s.	n.s.	n.s.
LSD 5 μg/kg	Not tested	$F(2,27)=16.94$, $p<0.001$	$F(2,27)=7.78$, $p=0.002$	$F(2,27)=11.42$, $p<0.001$	$F(2,27)=4.11$, $p=0.028$	n.s.
LSD 50 μg/kg	Not tested	$F(2,27)=3.44$, $p=0.047$	$F(2,27)=3.90$, $p=0.032$	n.s.	n.s.	n.s.
LSD 200 μg/kg	Not tested	$F(2,30)=3.51$, $p=0.043$	n.s.	$F(2,30)=3.58$, $p=0.04$	n.s.	$F(2,30)=9.57$, $p<0.001$

Total time spent rearing (Fig. 3A, Tables 1 and 3) was significantly affected by LSD treatment in all groups with post-hoc test showing a decrease in rearing following LSD 200 μg/kg in all three groups ($p<0.05$–$p<0.001$) when compared to their respective control group. In contrast rearing was increased in EP females after LSD 5 μg/kg ($p=0.05$).

An effect of LSD treatment on *total time spent sniffing the floor, walls and air* (Fig. 3B, Tables 1 and 3) was observed in all groups. Post-hoc tests showed that given the lowest LSD dose, only males displayed a significant decrease of sniffing ($p<0.05$). No change in sniffing was observed in animals given the medium LSD dose, in contrast a remarkable suppression of sniffing following the highest LSD dose in all three groups when compared to their respective controls was observed ($p<0.01$–$p<0.001$).

Total time spent grooming (Fig. 4A, Tables 1 and 3) was also affected in all groups tested. Post-hoc tests showed no changes in grooming after the two lower doses of LSD. In contrast, almost no grooming was measured in animals of all three groups given the highest LSD dose, however in post-hoc tests it reached significance only in male and MD female rats ($p<0.05$–$p<0.001$).

LSD significantly increased *total time spent immobile* (Fig. 4B, Tables 1 and 3) in all groups tested, however compared with the controls, no change in immobility time was found in animals given the lowest and the medium LSD doses. Using the highest LSD dose, even though an increase in immobility was obvious in all groups, the only significant one was in MD females ($p<0.001$) when compared to their controls.

Total time spent in a flat body posture (Tables 1 and 3) was increased in all groups after LSD with post-hoc analysis showing that only LSD 200 μg/kg increased the behaviour in all groups ($p<0.001$) compared to their controls.

Wet dog shakes (*total number of periods*) (Tables 1 and 3) occurred in all groups. While in post-hoc tests only EP females given the lowest LSD dose exhibited significantly more wet dog shakes than the controls ($p<0.05$), the medium LSD dose (50 μg/kg) significantly increased the occurrence of the pattern in all three groups of animals ($p<0.01$–$p<0.001$). When using the highest LSD dose, the number of periods was similar to the controls.

3.3. Prepulse inhibition (PPI) (Fig. 5, Table 1) of acoustic startle reaction (ASR) (Tables 1 and 4)

ASR was not altered by LSD treatment [$F(3,120)=1.62$, $p=0.19$], neither were there any group differences [$F(2,120)=0.49$, $p=0.61$] or treatment × groups interaction [$F(6,120)=0.72$, $p=0.63$]. One-way ANOVA has shown no treatment effects for any group (Tables 1 and 4).

Table 3
Two-way ANOVA and one-way ANOVA results of open field behaviour. n.s. means non significant.

Open field behaviour	Rearing	Sniffing	Grooming	Immobility	Flat body posture	Wet dog shakes
Two-way ANOVA						
Treatment factor	$FF(3,112)=29.43$, $p<0.001$	$F(3,112)=31.49$, $p<0.001$	$F(3,112)=11.44$, $p<0.001$	$F(3,112)=15.24$, $p<0.001$	$F(3,112)=82.63$, $p<0.001$	$F(3,112)=23.50$, $p<0.001$
Group factor	$F(2,112)=6.64$, $p=0.002$	$F(2,112)=4.91$, $p=0.009$	$F(2,112)=0.48$, $p=0.62$	$F(2,112)=5.05$ $p=0.008$	$F(2,112)=0.58$ $p=0.57$	$F(2,112)=0.95$, $p=0.39$
Treatment × group interaction	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
One-way ANOVA — treatment effect						
Males	$F(3,37)=5.84$, $p=0.002$	$F(3,37)=8.83$, $p<0.001$	$F(3,37)=6.11$, $p=0.002$	$F(3,37)=4.03$, $p=0.014$	$F(3,37)=22.19$, $p<0.001$	$F(3,37)=5.99$, $p=0.002$
EP females	$F(3,36)=11.23$, $p<0.001$	$F(3,36)=8.42$, $p<0.001$	$F(3,36)=3.00$ $p=0.044$	$F(3,36)=3.66$, $p=0.021$	$F(3,36)=15.06$, $p<0.001$	$F(3,36)=10.82$, $p<0.001$
MD females	$F(3,39)=14.06$, $p<0.001$	$F(3,39)=16.24$, $p<0.001$	$F(3,39)=4.76$, $p=0.006$	$F(3,39)=12.44$, $p<0.001$	$F(3,39)=66.59$, $p<0.001$	$F(3,39)=7.30$, $p<0.001$

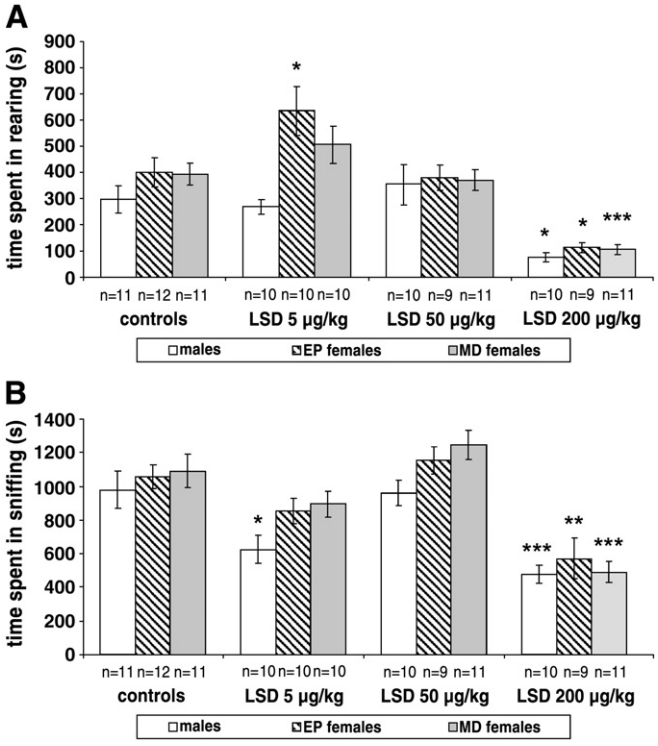

Fig. 3. (A) The effect of LSD on rearing. LSD 5 µg/kg increased rearing in EP female rats. LSD 200 µg/kg suppressed rearing in all three groups. (B) The effect of LSD on sniffing. LSD 5 µg/kg decreased sniffing in males but not in any female group. LSD 200 µg/kg decreased sniffing in all three groups. *, **, *** for $p<0.05$, 0.01 and 0.001 from respective control group; *n* under each column indicates the number of animals per group.

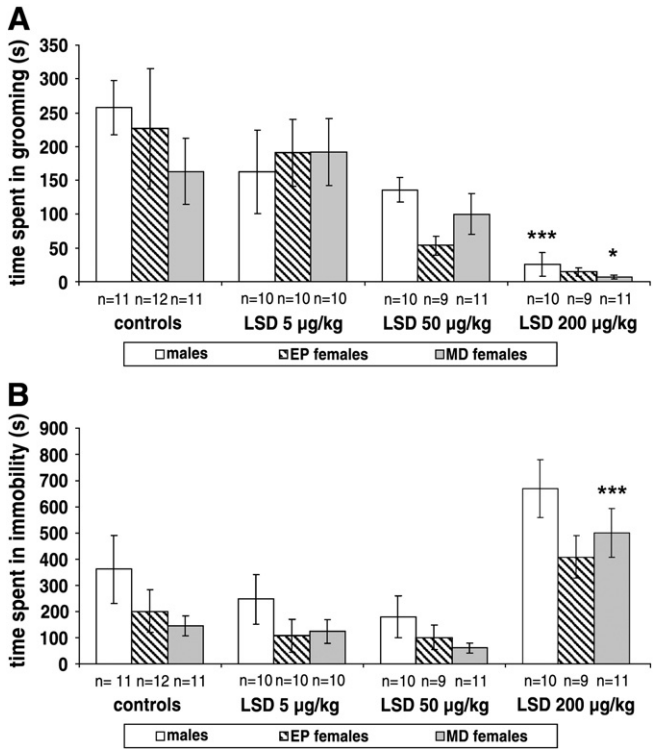

Fig. 4. (A) The effect of LSD on grooming. LSD 200 µg/kg significantly suppressed grooming in males and MD females. (B) The effect of LSD on immobility. LSD 200 µg/kg increased time spent immobile in all groups, however, only in MD females did it reach significance. *, *** for $p<0.05$ and 0.001 from respective control group; *n* under each column indicates the number of animals per group.

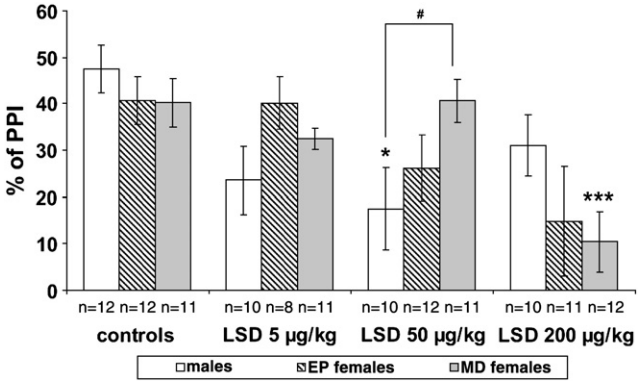

Fig. 5. The effect of LSD on PPI ASR. LSD 50 µg/kg disrupted PPI in male rats, LSD 200 µg/kg disrupted PPI in MD female group. LSD did not influence PPI in EP females. A sex difference was observed between MD females and males treated with LSD 50 µg/kg. * for $p<0.05$ and *** for $p<0.001$ from respective control group, # for $p<0.05$ between sex groups; *n* under each column indicates the number of animals per group.

PPI was significantly affected by treatment [$F(3,120)=7.11$, $p<0.001$] but not of groups [$F(2,120)=0.03$, $p=0.98$]. There was a significant treatment × groups interaction [$F(6,120)=2.51$, $p=0.026$].

One-way ANOVA for treatment effects showed significant disruption of PPI in males [$F(3,40)=3.02$, $p=0.041$] and MD females [$F(3,41)=10.11$, $p<0.001$] but not in EP females. Post-hoc analysis revealed that males treated with LSD 50 µg/kg ($p<0.05$) and MD females treated with LSD 200 µg/kg ($p<0.001$) had significantly disrupted PPI compared to their respective control groups.

One-way ANOVA between groups has shown no differences among the control animals, LSD 5 µg/kg and LSD 200 µg/kg, however, for LSD 50 µg/kg [$F(2,30)=3.33$, $p=0.049$] MD females had significantly higher PPI than males as shown by post-hoc analysis ($p<0.05$).

4. Discussion

LSD altered behavioural parameters in all of the tests performed. Although we did not find statistically significant differences between oestral phases in females, we observed that EP females were protected from some of the behavioural effects of LSD. Interestingly, during the cycle the highest oestradiol and progesterone levels in females are achieved in pro-oestrus and oestrus (Smith, 1994). On the contrary we also observed that in some experiments both EP and MD females had similar reactions when compared to males. These findings highlight the role of both acute as well as long-lasting effects of female sex steroids on the behavioural action of LSD. To our knowledge, there are no studies with psychedelics in rats that compared sex differences in their effects. As mentioned previously, the main action of LSD is thought to be governed by serotonin 5-$HT_{2A/C}$ and 5-HT_{1A} receptors (Nichols et al., 2002). It has been shown in many other studies that serotonin 5-$HT_{2A/C}$ and 5-HT_{1A} receptors are strongly influenced by female steroid hormones. In general, evidence supports the theory that oestrogens and progesterone increase density/expression and/or sensitivity of 5-$HT_{2A/C}$ and 5-HT_{1A} receptors (Birzniece et al., 2002; Blanchard et al., 1992; Cyr et al., 2000; Fink et al., 1999; Frankfurt et al.,

Table 4
The effect of LSD on acoustic startle response. Table shows magnitude of ASR. No significance was observed in ASR in any group.

	ASR amplitude (±SEM)			
	Controls	LSD 5 µg/kg	LSD 50 µg/kg	LSD 200 µg/kg
Males	71.33 (±9.69)	51.38 (±10.25)	69.58 (±12.85)	56.43 (±10.82)
EP females	68.6 (±9.32)	81.45 (±17.58)	76.65 (±16.38)	52.51 (±7.73)
MD females	70.2 (±14.17)	75.89 (±15.05)	59.18 (±9.95)	51.43 (±5.69)

1994; Haleem et al., 1990; Landry and Di, 2003; Lanfumey and Hamon, 2004; Le Saux and Di Paolo, 2005; Nakano et al., 1992; Summer and Fink, 1995, 1998; Sumner et al., 2007; Zhou et al., 2002). Based on these studies we might expect sex hormones to play an important role in the sensitivity of the serotonergic system to LSD's behavioural effects as shown above. However, further studies are needed to explore specific receptor–steroid interactions which might underlie the observed effects.

4.1. Locomotor activity

LSD induced an initial hypolocomotion in males and MD females. Interestingly, EP females in our setting were not affected by the hypolocomotor effects of LSD, on the contrary a slight increase in locomotion was observed between 30 and 45 min after administration. The hypolocomotor effect can be related to initial ataxia and has been previously described in LSD as well as other hallucinogens (Krebs-Thomson et al., 1998, 2006; Krebs-Thomson and Geyer, 1996; Palenicek et al., 2006, 2008; Shah and Hedden, 1978; Sykes, 1986). On the contrary increased locomotion in EP females during the second part of the testing period indicates attenuation of habituation (Lát, 1973; Wishaw et al., 1999). Comparable to our work, Kabes and Fink (1969) found that in both male and female rats, irrespective of sex, the maximal hypolocomotor effects of LSD appeared at 20–30 min after administration. Other studies have described a biphasic temporal effect of LSD, however, there are several discrepancies between the findings. LSD 200 μg/kg in male rats induced an initial increase in spontaneous motor activity followed by a long-lasting decrease in a study performed by Kabes et al. (1972), yet conversely initial inhibition followed by stimulation in LSD and other hallucinogens has also been described (Geyer et al., 1979). Finally, some studies also reported sole hyperlocomotor effects of LSD (Mittman and Geyer, 1991).

The low and intermediate LSD doses led both EP and MD females to significantly higher probabilities than males appearing in the centre of the arena (lower thigmotaxis). This higher appearance of rats in central parts of an open field can be associated with increased exploration (Lát, 1973; Wishaw et al., 1999) caused by LSD. This is also supported by increased rearing in EP females. Congruent with our findings in males, LSD and another hallucinogen 5-methoxy-N, N-dimethyltryptamine (5-MeO-DMT) have also been reported to decrease entrance to central parts of the arena in male rats (Adams and Geyer, 1985a,1985b) and even in females (Kabes and Fink, 1971) in other studies.

4.2. Behavioural repertoire in the open field test

The results obtained in the qualitative behavioural profile of LSD are summarized in Table 1. The higher the LSD dose the more clear-cut the impairment of the spontaneous behavioural repertoire of rats. In fact, exploratory patterns such as sniffing and rearing as well as grooming patterns disappear and the immobility of animals increases and is accompanied with flat body postures and wet dog shakes. On the contrary, a significant increase in rearing (exploratory behaviour) following the lowest LSD dose was found in EP females. A comparable pattern of changes with LSD doses of 60 to 200 μg/kg in male rats regarding the disappearance of normal behaviour like grooming or rearing and an increase in inactivity has also been described previously (Adams and Geyer, 1985a; Kabes et al., 1972; Kabes and Fink, 1969, 1971; Krebs-Thomson et al., 1998). Flat body posture and wet dog shakes (and/or head waving/twitching in other studies) after various serotonergic drugs are often mentioned as constituents of behavioural serotonin syndrome and/or as a marker of stereotypy (Fantegrossi et al., 2008; Fone et al., 1989; Oekelen et al., 2002; Pranzatelli and Pluchino, 1991; Yamamoto and Ueki, 1981).

4.3. Sex differences in open field behavioural parameters

Sex differences observed in locomotion and open field behavioural repertoire can be summarized as follows: 1) increased locomotion and/or lower sensitivity to hypolocomotor changes in EP and MD female rats treated with low doses of LSD, 2) decreased thigmotaxis following treatment with low and intermediate doses of LSD in EP and MD females compared to males, and 3) increased rearing after the lowest LSD dose in EP females but not in males or MD females.

A protective effect of E2 and progesterone on hypolocomotion especially in EP females is important. It has been shown that female steroid hormones protect against dyskinesia induced by LSD (Selye, 1971). Congruently, during the cycle females in pro-oestrus and oestrus have the highest E2 and progesterone levels (Smith, 1994) as mentioned above. Further to support this hypothesis, E2 administration to the median raphe nucleus increased locomotion in ovariectomized females (Andrade et al., 2005), and in another study E2 decreased the immobility of rats (Dhir and Kulkarni, 2008).

It is most likely that differences between the oestral phases in the regulation of serotonin 5-HT2$_{A/C}$ and 5-HT$_{1A}$ receptors, on which LSD has a primary action, underlie these effects. In our previous studies we have found that hypolocomotion induced by hallucinogens is blocked by selective 5-HT$_{2C}$ receptor antagonists (Kutova et al., 2008; Palenicek et al., 2007a). Thus regulation of the 5-HT$_{2C}$ receptor by sex steroids (Zhou et al., 2002) might underlie these changes.

Increased rearing and lower thigmotaxis in EP, and less in MD females, are probably linked to increased exploratory activity and possibly also decreased anxiety (Wishaw et al., 1999). It is of interest that even though the differences between the groups failed to reach statistical significance, we saw almost double the time spent in rearing in EP females compared to males. A similar increase in rearing was observed in our previous studies with MDMA (Palenicek et al., 2005, 2007b). Thus some common mechanisms between these two serotonergic drugs, even if they do not completely share the same mechanism of action, might be expected. One possible explanation is the involvement of dopamine D$_1$ receptors. In other studies it has been shown that stimulation of these receptors increased rearing in male rats (Deveney and Waddington, 1995; Ikemoto, 2002; Meyer and Shults, 1993). LSD, apart from its action on serotonin receptors, also acts as an agonist of D$_1$ receptors (Watts et al., 1995), similarly, MDMA stimulates dopamine release (Lyles and Cadet, 2003; Slikker Jr. et al., 1989) and dopaminergic system functions are known to be altered by female sex steroids (Attali et al., 1997; Bazzett and Becker, 1994; Bosse et al., 1997; Zhou et al., 2002).

4.4. Prepulse inhibition (PPI) of acoustic startle reaction (ASR)

LSD disrupted PPI in males and MD females, while in EP females its effect failed to reach statistical significance, even though PPI was markedly reduced after the LSD 200 μg/kg. The suppression of PPI is in congruence with other studies on LSD and other psychedelics (Krebs-Thomson et al., 2006; Ouagazzal et al., 2001; Palenicek et al., 2008; Sipes and Geyer, 1995). The receptor mechanisms are generally linked to the agonistic action of LSD at 5-HT$_{2A}$ receptors (Leng et al., 2003; Ouagazzal et al., 2001; Padich et al., 1996; Sipes and Geyer, 1994, 1995, 1997). It is surprising that despite LSD being a powerful psychedelic in extremely low doses in humans, it did not significantly alter PPI in lower doses in our setting. One plausible explanation is that with respect to the relatively long duration of the effects of LSD (Kabes et al., 1972; Marona-Lewicka et al., 2005; Marona-Lewicka and Nichols, 2007), the disruption might occur later in all groups and LSD treatments, as was similarly observed with mescaline (Palenicek et al., 2008). On the contrary, others have observed a dose dependent disruption of PPI with LSD in a similar temporal arrangement (Ouagazzal et al., 2001).

4.5. Sex differences in PPI

Analysis of the effects of LSD on PPI revealed several differences between males and females. In males only an intermediate dose significantly disrupted PPI, while in MD females only the highest dose did so. On the contrary, EP females were not significantly affected. Thus, LSD-treated EP females showed lower sensitivity to the disruptive effects of LSD on PPI. Similar findings were found in a previous study with MDMA (Bubenikova et al., 2005), again possibly underlying similar mechanisms. Since deficits in PPI induced by LSD are predominantly assigned to its 5-HT_{2A} agonistic action (Leng et al., 2003; Ouagazzal et al., 2001; Padich et al., 1996; Sipes and Geyer, 1994, 1995, 1997), the discrepancies observed between male and female rats and LSD dosage in our setting can be related to long-lasting oestrogen/progesterone effects on these receptors. As shown by others, female sex steroids increase expression/density of 5-HT_{2A} receptors (Birzniece et al., 2002; Cyr et al., 2000; Fink et al., 1999; Summer and Fink, 1995, 1998; Sumner et al., 2007; Zhou et al., 2002). Since levels of these hormones are highest during natural pro-oestrus and oestrus it is plausible, that females have a bigger pool of 5-HT_{2A} receptors during these phases, thus higher doses of 5-HT_{2A} agonists are probably needed to disrupt prepulse inhibition.

5. Conclusion

We have observed that LSD disrupted the behavioural repertoire and sensorimotor gating in both sexes. The main sex differences observed were: lower sensitivity of females to the hypolocomotor effects of LSD and an increase in locomotion in EP females, increased rearing behaviour in EP females, lower thigmotaxis in both female groups of rats after the lowest and intermediate LSD treatment. Finally, we observed decreased sensitivity of EP females and lower sensitivity and/or different dose effectiveness in MD females in the disruptive effects of LSD on PPI. In general, we can conclude that the oestral and pro-oestral phases of the female cycle protected against some of the effects of LSD. A suppression of normal behaviour and appearance of atypical behaviour with intermediate and high LSD treatment was not explicitly affected by sex. Observed sex differences are probably linked to both acute- and long-lasting sex steroid related regulation of serotonin and dopamine systems. In conclusion, sex, and even the female oestral cycle, plays an important role in the manifestation of the behavioural effects of hallucinogens. Since hallucinogens are used as serotonergic models of psychosis, it also opens important future issues for studying these models and atypical antipsychotic action between sexes.

Acknowledgments

This work was supported by the grants of the Czech Ministry of Health IGA MHCR NS 10375-3, NS 10374-3, NR 9324-3 and by the grants CNS MEYS CR 1M0517 and MZ0PCP2005. We thank Ingmar Gorman, BA and Craig Hampson BSc (Hons) for helpful comments and language correction.

References

Abel KM, Allin MP, Hemsley DR, Geyer MA. Low dose ketamine increases prepulse inhibition in healthy men. Neuropharmacology 2003;44:729–37.
Adams LM, Geyer MA. A proposed animal model for hallucinogens based on LSD's effects on patterns of exploration in rats. Behav Neurosci 1985a;99:881–900.
Adams LM, Geyer MA. Effects of DOM and DMT in a proposed animal model of hallucinogenic activity. Prog Neuropsychopharmacol Biol Psychiatry 1985b;9:121–32.
Allott K, Redman J. Are there sex differences associated with the effects of ecstasy/3, 4-methylenedioxymethamphetamine (MDMA)? Neurosci Biobehav Rev 2007;31: 327–47.
Andrade TG, Nakamuta JS, Avanzi V, Graeff FG. Anxiolytic effect of estradiol in the median raphe nucleus mediated by 5-HT1A receptors. Behav Brain Res 2005;163:18–25.
Attali G, Weizman A, Gil-Ad I, Rehavi M. Opposite modulatory effects of ovarian hormones on rat brain dopamine and serotonin transporters. Brain Res 1997;756: 153–9.
Bazzett TJ, Becker JB. Sex differences in the rapid and acute effects of estrogen on striatal D2 dopamine receptor binding. Brain Res 1994;637:163–72.
Birzniece V, Johansson IM, Wang MD, Backstrom T, Olsson T. Ovarian hormone effects on 5-hydroxytryptamine(2A) and 5-hydroxytryptamine(2C) receptor mRNA expression in the ventral hippocampus and frontal cortex of female rats. Neurosci Lett 2002;319:157–61.
Blanchard DC, Shepherd JK, Rodgers RJ, Blanchard RJ. Evidence for differential effects of 8-OH-DPAT on male and female rats in the anxiety/defense test battery. Psychopharmacology (Berl) 1992;106:531–9.
Bosse R, Rivest R, Di Paolo T. Ovariectomy and estradiol treatment affect the dopamine transporter and its gene expression in the rat brain. Brain Res Mol Brain Res 1997;46: 343–6.
Bubenikova V, Votava M, Horacek J, Palenicek T. Relation of sex and estrous phase to deficits in prepulse inhibition of the startle response induced by ecstasy (MDMA). Behav Pharmacol 2005;16:127–30.
Carroll ME, Lynch WJ, Roth ME, Morgan AD, Cosgrove KP. Sex and estrogen influence drug abuse. Trends Pharmacol Sci 2004;25:273–9.
Cosgrove KP, Mazure CM, Staley JK. Evolving knowledge of sex differences in brain structure, function, and chemistry. Biol Psychiatry 2007;62:847–55.
Cunha JM, Masur J. Evaluation of psychotropic drugs with a modified open field test. Pharmacology 1978;16:259–67.
Cyr M, Landry M, Di PT. Modulation by estrogen-receptor directed drugs of 5-hydroxytryptamine-2A receptors in rat brain. Neuropsychopharmacology 2000;23: 69–78.
Deveney AM, Waddington JL. Pharmacological characterization of behavioural responses to SK&F 83959 in relation to 'D1-like' dopamine receptors not linked to adenylyl cyclase. Br J Pharmacol 1995;116:2120–6.
Dhir A, Kulkarni SK. Antidepressant-like effect of 17beta-estradiol: involvement of dopaminergic, serotonergic, and (or) sigma-1 receptor systems. Can J Physiol Pharmacol 2008;86:726–35.
Egan CT, Herrick-Davis K, Miller K, Glennon RA, Teitler M. Agonist activity of LSD and lisuride at cloned 5HT2A and 5HT2C receptors. Psychopharmacology (Berl) 1998;136:409–14.
Fantegrossi WE, Reissig CJ, Katz EB, Yarosh HL, Rice KC, Winter JC. Hallucinogen-like effects of N, N-dipropyltryptamine (DPT): possible mediation by serotonin 5-HT1A and 5-HT2A receptors in rodents. Pharmacol Biochem Behav 2008;88:358–65.
Fink G, Sumner B, Rosie R, Wilson H, McQueen J. Androgen actions on central serotonin neurotransmission: relevance for mood, mental state and memory. Behav Brain Res 1999;105:53–68.
Fink G, Sumner BE, Rosie R, Grace O, Quinn JP. Estrogen control of central neurotransmission: effect on mood, mental state, and memory. Cell Mol Neurobiol 1996;16:325–44.
Fone KC, Johnson JV, Bennett GW, Marsden CA. Involvement of 5-HT2 receptors in the behaviours produced by intrathecal administration of selected 5-HT agonists and the TRH analogue (CG 3509) to rats. Br J Pharmacol 1989;96:599–608.
Frankfurt M, McKittrick CR, Mendelson SD, McEwen BS. Effect of 5, 7-dihydroxytryptamine, ovariectomy and gonadal steroids on serotonin receptor binding in rat brain. Neuroendocrinology 1994;59:245–50.
Fu CH, Abel KM, Allin MP, Gasston D, Costafreda SG, Suckling J, et al. Effects of ketamine on prefrontal and striatal regions in an overt verbal fluency task: a functional magnetic resonance imaging study. Psychopharmacology (Berl) 2005;183:92-102.
Geyer MA, Light RK, Rose GJ, Petersen LR, Horwitt DD, Adams LM, et al. A characteristic effect of hallucinogens on investigatory responding in rats. Psychopharmacology (Berl) 1979;65:35–40.
Gouzoulis-Mayfrank E, Heekeren K, Neukirch A, Stoll M, Stock C, Obradovic M, et al. Psychological effects of (S)-ketamine and N, N-dimethyltryptamine (DMT): a double-blind, cross-over study in healthy volunteers. Pharmacopsychiatry 2005;38:301–11.
Grailhe R, Waeber C, Dulawa SC, Hornung JP, Zhuang X, Brunner D, et al. Increased exploratory activity and altered response to LSD in mice lacking the 5-HT(5A) receptor. Neuron 1999;22:581–91.
Haleem DJ, Kennett GA, Curzon G. Hippocampal 5-hydroxytryptamine synthesis is greater in female rats than in males and more decreased by the 5-HT1A agonist 8-OH-DPAT. J Neural Transm Gen Sect 1990;79:93-101.
Hasler F, Grimberg U, Benz MA, Huber T, Vollenweider FX. Acute psychological and physiological effects of psilocybin in healthy humans: a double-blind, placebo-controlled dose–effect study. Psychopharmacology (Berl) 2004;172:145–56.
Hermle L, Funfgeld M, Oepen G, Botsch H, Borchardt D, Gouzoulis E, et al. Mescaline-induced psychopathological, neuropsychological, and neurometabolic effects in normal subjects: experimental psychosis as a tool for psychiatric research. Biol Psychiatry 1992;32: 976–91.
Horowski R. Pharmacological effects of lisuride and their potential role in further research. In: Calne DB, McDonald RJ, Horowski R, Wuttke W, editors. Lisuride and other dopamine agonists — basic mechanisms and endocrine and neurological effects. New York: Raven Press; 1983. p. 127–39.
Horowski R, Wachtel H. Pharmacological effects of lisuride in rodents mediated by dopaminergic receptors: mechanisms of action and influence of chronic treatment with lisuride. In: Fuxe K, Calne DB, editors. Oxford, New York: Pergamon Press; 1979. p. 237–51.
Ikemoto S. Ventral striatal anatomy of locomotor activity induced by cocaine, D-amphetamine, dopamine and D1/D2 agonists. Neuroscience 2002;113:939–55.
Kabes J, Fink Z. Alteration of some patterns of spontaneous behavior in rats after LSD. Act Nerv Super (Praha) 1971;13:99-100.
Kabes J, Fink Z. Spontaneous motor activity and acetylcholine brain metabolism in rats after LSD administration. Basel: Schwabe; 1969. p. 73–4.
Kabes J, Fink Z, Roth Z. A new device for measuring spontaneous motor activity—effects of lysergic acid diethylamide in rats. Psychopharmacologia 1972;23:75–85.
Koch M. The neurobiology of startle. Prog Neurobiol 1999;59:107–28.

Krebs-Thomson K, Geyer MA. The role of 5-HT(1A) receptors in the locomotor-suppressant effects of LSD: WAY-100635 studies of 8-OH-DPAT, DOI and LSD in rats. Behav Pharmacol 1996;7:551–9.
Krebs-Thomson K, Paulus MP, Geyer MA. Effects of hallucinogens on locomotor and investigatory activity and patterns: influence of 5-HT2A and 5-HT2C receptors. Neuropsychopharmacology 1998;18:339–51.
Krebs-Thomson K, Ruiz EM, Masten V, Buell M, Geyer MA. The roles of 5-HT(1A) and 5-HT (2) receptors in the effects of 5-MeO-DMT on locomotor activity and prepulse inhibition in rats. Psychopharmacology (Berl) 2006;189:319–29.
Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, et al. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch Gen Psychiatry 1994;51: 199–214.
Kutova M, Fujakova M, Palenicek T, Bubenikova-Valesova V. Srovnání účinku psychedelik mezkalinu a 2C-B vanimálním modelu - role serotoninových 5-HT2 a 5-HT1 receptorů (Comparison of the effects of psychedelics mescaline and 2C-B in animal model — role of serotonin 5-HT2 and 5-HT1 receptors) (Abstract). Psychiatrie 2008;12:47.
Landry M, Di PT. Effect of chronic estradiol, tamoxifen or raloxifene treatment on serotonin 5-HT1A receptor. Brain Res Mol Brain Res 2003;112:82–9.
Lanfumey L, Hamon M. 5-HT1 receptors. Curr Drug Targets CNS Neurol Disord 2004;3: 1-10.
Lát J. The analysis of habituation. Acta Neurobiol Exp (Wars) 1973;33:771–89.
Le Saux M, Di Paolo T. Changes in 5-HT1A receptor binding and G-protein activation in the rat brain after estrogen treatment: comparison with tamoxifen and raloxifene. J Psychiatry Neurosci 2005;30:110–7.
Lees J, Hallak JE, Deakin JF, Dursun SM. Gender differences and the effects of ketamine in healthy volunteers. J Psychopharmacol 2004;18:337–9.
Leng A, Ouagazzal A, Feldon J, Higgins GA. Effect of the 5-HT6 receptor antagonists Ro04-6790 and Ro65-7199 on latent inhibition and prepulse inhibition in the rat: comparison to clozapine. Pharmacol Biochem Behav 2003;75:281–8.
Liechti ME, Gamma A, Vollenweider FX. Gender differences in the subjective effects of MDMA. Psychopharmacology (Berl) 2001;154:161–8.
Lyles J, Cadet JL. Methylenedioxymethamphetamine (MDMA, ecstasy) neurotoxicity: cellular and molecular mechanisms. Brain Res Brain Res Rev 2003;42:155–68.
Marcondes FK, Bianchi FJ, Tanno AP. Determination of the estrous cycle phases of rats: some helpful considerations. Braz J Biol 2002;62:609–14.
Marek GJ, Aghajanian GK. Indoleamine and the phenethylamine hallucinogens: mechanisms of psychotomimetic action. Drug Alcohol Depend 1998;51:189–98.
Marek GJ, Aghajanian GK. LSD and the phenethylamine hallucinogen DOI are potent partial agonists at 5-HT2A receptors on interneurons in rat piriform cortex. J Pharmacol Exp Ther 1996;278:1373–82.
Marona-Lewicka D, Nichols DE. Further evidence that the delayed temporal dopaminergic effects of LSD are mediated by a mechanism different than the first temporal phase of action. Pharmacol Biochem Behav 2007;87:453–61.
Marona-Lewicka D, Thisted RA, Nichols DE. Distinct temporal phases in the behavioral pharmacology of LSD: dopamine D2 receptor-mediated effects in the rat and implications for psychosis. Psychopharmacology (Berl) 2005;180:427–35.
McCann UD, Ridenour A, Shaham Y, Ricaurte GA. Serotonin neurotoxicity after (+/−)3, 4-methylenedioxymethamphetamine (MDMA; "Ecstasy"): a controlled study in humans. Neuropsychopharmacology 1994;10:129–38.
Meehan SM, Schechter MD. LSD produces conditioned place preference in male but not female fawn hooded rats. Pharmacol Biochem Behav 1998;59:105–8.
Meyer ME, Shults JM. Dopamine D1 receptor family agonists, SK&F38393, SK&F77434, and SK&F82958, differentially affect locomotor activities in rats. Pharmacol Biochem Behav 1993;46:269–74.
Mittman SM, Geyer MA. Dissociation of multiple effects of acute LSD on exploratory behavior in rats by ritanserin and propranolol. Psychopharmacology (Berl) 1991;105: 69–76.
Monroe RR, Heath RG, Mickle WA, Llewellyn RC. Correlation of rhinencephalic electrograms with behavior; a study on humans under the influence of LSD and mescaline. Electroencephalogr Clin Neurophysiol 1957;9:623–42.
Nakano Y, Matsuda T, Takuma K, Yoshikawa T, Baba A. Sex difference for tolerance of 5-HT1A receptor-mediated temperature and corticosterone responses in mice. Eur J Pharmacol 1992;219:339–41.
Nichols DE, Frescas S, Marona-Lewicka D, Kurrasch-Orbaugh DM. Lysergamides of isomeric 2, 4-dimethylazetidines map the binding orientation of the diethylamide moiety in the potent hallucinogenic agent N, N-diethyllysergamide (LSD). J Med Chem 2002;45:4344–9.
Ouagazzal A, Grottick AJ, Moreau J, Higgins GA. Effect of LSD on prepulse inhibition and spontaneous behavior in the rat. A pharmacological analysis and comparison between two rat strains. Neuropsychopharmacology 2001;25:565–75.
Padich RA, McCloskey TC, Kehne JH. 5-HT modulation of auditory and visual sensorimotor gating: II. effects of the 5-HT2A antagonist MDL 100, 907 on disruption of sound and light prepulse inhibition produced by 5-HT agonists in Wistar rats. Psychopharmacology (Berl) 1996;124:107–16.
Palenicek T, Bubenikova V, Votava M, Horacek J. Účinky selektivního antagonisty serotoninového 5-HT_{2C} receptoru SB242084 na lokomoci potkana v animálních modelech psychóz (The effects of selective antagonist of serotonin 5-HT_{2C} receptor SB242084 on rat`s locomotion in animal models of psychosis). Adiktologie 2006;10: 16–9.
Palenicek T, Balikova M, Bubenikova-Valesova V, Horacek J. Mescaline effects on rat behavior and its time profile in serum and brain tissue after a single subcutaneous dose. Psychopharmacology (Berl) 2008;196:51–62.
Palenicek T, Bubenikova-Valesova V, Horacek J. The role of serotonin 5-HT2A/C receptors in the behavioral action of the synthetic drug 2C-B (Abstract). World J Biol Psychiatry 2007a;8:205.
Palenicek T, Hlinak Z, Bubenikova-Valesova V, Votava M, Horacek J. An analysis of spontaneous behavior following acute MDMA treatment in male and female rats. Neuro Endocrinol Lett 2007b;28:781–8.
Palenicek T, Votava M, Bubenikova V, Horacek J. Increased sensitivity to the acute effects of MDMA ("ecstasy") in female rats. Physiol Behav 2005;86:546–53.
Pranzatelli MR, Pluchino RS. The relation of central 5-HT1A and 5-HT2 receptors: low dose agonist-induced selective tolerance in the rat. Pharmacol Biochem Behav 1991;39:407–13.
Reneman L, Booij J, De Bruin K, Reitsma JB, de Wolff FA, Gunning WB, et al. Effects of dose, sex, and long-term abstention from use on toxic effects of MDMA (ecstasy) on brain serotonin neurons. Lancet 2001;358:1864–9.
Roth ME, Cosgrove KP, Carroll ME. Sex differences in the vulnerability to drug abuse: a review of preclinical studies. Neurosci Biobehav Rev 2004;28:533–46.
Roubicek J, Srnec J. Experimental psychosis caused by LSD. Cas Lek Cesk 1955;94:189–95.
Schwarz BE, Sem-Jacobsen CW, Petersen MC. Effects of mescaline, LSD-25, and adrenochrome on depth electrograms in man. AMA Arch Neurol Psychiatry 1956;75:579–87.
Seeman P, Guan HC, Hirbec H. Dopamine D2(High) receptors stimulated by phencyclidines, lysergic acid diethylamide, salvinorin A, and modafinil. Synapse 2009;63: 698–704.
Selye H. Protection against LSD by various steroids. Life Sci 1971;10:1135–40.
Shah NS, Hedden MP. Behavioral effects and metabolic fate of N, N-dimethyltryptamine in mice pretreated with beta-diethylaminoethyl-diphenylpropylacetate (SKF 525-A), iproniazid and chlorpromazine. Pharmacol Biochem Behav 1978;8:351–6.
Sipes TA, Geyer MA. Multiple serotonin receptor subtypes modulate prepulse inhibition of the startle response in rats. Neuropharmacology 1994;33:441–8.
Sipes TE, Geyer MA. DOI disruption of prepulse inhibition of startle in the rat is mediated by 5-HT(2A) and not by 5-HT(2C) receptors. Behav Pharmacol 1995;6: 839–42.
Sipes TE, Geyer MA. DOI disrupts prepulse inhibition of startle in rats via 5-HT2A receptors in the ventral pallidum. Brain Res 1997;761:97-104.
Slikker Jr W, Holson RR, Ali SF, Kolta MG, Paule MG, Scallet AC, et al. Behavioral and neurochemical effects of orally administered MDMA in the rodent and nonhuman primate. Neurotoxicology 1989;10:529–42.
Smith SS. Female sex steroid hormones: from receptors to networks to performance—actions on the sensorimotor system. Prog Neurobiol 1994;44:55–86.
Summer BE, Fink G. Estrogen increases the density of 5-hydroxytryptamine(2A) receptors in cerebral cortex and nucleus accumbens in the female rat. J Steroid Biochem Mol Biol 1995;54:15–20.
Sumner BE, Fink G. Testosterone as well as estrogen increases serotonin2A receptor mRNA and binding site densities in the male rat brain. Brain Res Mol Brain Res 1998;59:205–14.
Sumner BE, Grant KE, Rosie R, Hegele-Hartung C, Fritzemeier KH, Fink G. Raloxifene blocks estradiol induction of the serotonin transporter and 5-hydroxytryptamine2A receptor in female rat brain. Neurosci Lett 2007;417:95–9.
Swerdlow NR, Geyer MA, Braff DL. Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. Psychopharmacology (Berl) 2001;156:194–215.
Sykes EA. Mescaline-induced motor impairment in rats, assessed by two different methods. Life Sci 1986;39:1051–8.
van Oekelen D, Megens A, Meert T, Luyten WH, Leysen JE. Role of 5-HT(2) receptors in the tryptamine-induced 5-HT syndrome in rats. Behav Pharmacol 2002;13:313–8.
Vollenweider FX, Csomor PA, Knappe B, Geyer MA, Quednow BB. The effects of the preferential 5-HT2A agonist psilocybin on prepulse inhibition of startle in healthy human volunteers depend on interstimulus interval. Neuropsychopharmacology 2007;32:1876–87.
Vollenweider FX, Vollenweider-Scherpenhuyzen MF, Babler A, Vogel H, Hell D. Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. NeuroReport 1998;9:3897–902.
Watts VJ, Lawler CP, Fox DR, Neve KA, Nichols DE, Mailman RB. LSD and structural analogs: pharmacological evaluation at D1 dopamine receptors. Psychopharmacology (Berl) 1995;118:401–9.
Wishaw IQ, Haun F, Kolb B. Analysis of behavior in laboratory rodents. In: Windhorst U, Johansson H, editors. Modern techniques in neuroscience. Berlin, Germany: Springer-Verlag; 1999. p. 1243–75.
Wolbach Jr AB, Miner EJ, Isbell H. Comparison of psilocin with psilocybin, mescaline and LSD-25. Psychopharmacologia 1962;3:219–23.
Yamamoto T, Ueki S. The role of central serotonergic mechanisms on head-twitch and backward locomotion induced by hallucinogenic drugs. Pharmacol Biochem Behav 1981;14:89–95.
Zhou W, Cunningham KA, Thomas ML. Estrogen regulation of gene expression in the brain: a possible mechanism altering the response to psychostimulants in female rats. Brain Res Mol Brain Res 2002;100:75–83.