Pharmacology, Biochemistry and Behavior 98 (2011) 241–249

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Prenatal lipopolysaccharide treatment enhances MK-801-induced psychotomimetic effects in rats

A. Basta-Kaim [a,*], K. Fijał [b], B. Budziszewska [a], M. Regulska [a], M. Leśkiewicz [a], M. Kubera [a], K. Gołembiowska [c], W. Lasoń [a], K. Wędzony [b]

[a] Department of Experimental Neuroendocrinology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland
[b] Laboratory of Pharmacology and Brain Biostructure, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland
[c] Department of Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

ARTICLE INFO

Article history:
Received 12 April 2010
Received in revised form 17 December 2010
Accepted 23 December 2010
Available online 12 January 2011

Keywords:
Neurodevelopmental model of schizophrenia
Prepulse inhibition
Locomotor activity
MK-801

ABSTRACT

The aim of this study was to evaluate the effect of prenatal lipopolysaccharide (LPS) treatment, which is an animal developmental model of schizophrenia, on MK-801-induced psychotomimetic behavioral changes and brain aminergic system activity in adult offspring. Repeated LPS (1 mg/kg) injection in rats, that had started from 7th day of pregnancy and was continued every second day till delivery, resulted in a long-lasting disruption of prepulse inhibition (PPI) and elevation of locomotor activity in their offspring. The prenatally LPS-treated rats showed hypersensitivity to MK-801 (0.1 and 0.4 mg/kg) as evidenced by the enhancement of acoustic startle amplitude, reduced PPI, and enhanced locomotor activity.
These behavioral changes were accompanied by a decrease in the dopamine and its metabolite, DOPAC concentration in the frontal cortex, enhanced dopaminergic system activity in the striatum and no changes in noradrenaline (NA) level. Furthermore, the significant augmentation of 5-HT and 5-HIAA content in the frontal cortex of females only was detected. No changes in the cortical NA tissue level were found. Summing up, the present study demonstrated that the activation of the immune system in prenatal period led to persistent behavioral hypersensitivity to psychotomimetic action of a non-competitive NMDA receptor antagonist, and attention/information processing deficits. The foregoing data indicate that prenatal administration of LPS model some of the clinical aspects of schizophrenia and these behavioral effects are connected with neurochemical changes.

1. Introduction

Schizophrenia is regarded to be a multifactor brain disorder of neurodevelopmental origin, in which both genetic and environmental factors seem to be equally involved. Population and epidemiological studies have shown that maternal viruses or bacterial infection during pregnancy are associated with an increased risk of schizophrenia in offspring (Mednick et al., 1988; O'Callaghan et al., 1994; Brown et al., 2004). Furthermore, a systemic administration of the bacterial endotoxin lipopolysaccharide (LPS) has been proposed as a model of the schizophrenic symptoms of developmental origin (Borrell et al., 2002, Fortier et al., 2007, Romero et al., 2008). Indeed, perinatal administration of LPS, polyriboinosinic-polyribocytidilic acid (poly I:C) or inflammatory cytokine in rodents induced behavioral abnormalities which resemble those occurring in schizophrenic patients, such as changes in general motor activity, deficits in social interactions and impairments in sensorimotor gating (prepulse inhibition) as well as disturbances in body temperature, sleep and neuroendocrine function (Shi et al., 2003; Zuckerman and Weiner, 2005; Tohmi et al., 2004; Pauli et al., 1998; Pollmächer et al., 1993). The ability of LPS to induce the schizophrenia-like symptoms may be connected with the stimulation of peripheral immunoregulatory cytokine synthesis and release or enhancement of cytokine expression in the central nervous system by this endotoxin (Borrell et al., 2002; Romero et al., 2008). Abnormal sustainedly elevated levels of cytokines in the developing central nervous system may, in turn, lead to the changes in differentiation, migration, apoptosis, synaptogenesis or morphology of neural cells, and, in consequences, to the alterations in dopaminergic, GABA-ergic and glutamatergic transmission in adult offspring (Baharnoori et al., 2009; Muller and Schwarz, 2006; Nawa et al., 2000; Cai et al., 2000; Watanabe et al., 2004). Also recent data have put an emphasis on the role of dysregulation of the immune-mediated glutamatergic-dopaminergic neurotransmission in the patomechanism of schizophrenia. Uncompetitive NMDA receptor antagonists, such as ketamine, phencyclidine or MK-801 evoke effects resembling positive and negative symptoms of

* Corresponding author. Department of Experimental Neuroendocrinology, Institute of Pharmacology PAS, 12 Smetna St. PL31-343 Krakow, Poland. Tel.: +48 12 662 32 73; fax: +48 12 637 45 00.
E-mail address: basta@if-pan.krakow.pl (A. Basta-Kaim).

0091-3057/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2010.12.026

schizophrenia both in humans and animals (Krystal et al., 1994; Javitt and Zukin, 1991; Becker et al., 2003; Wędzony et al., 2008). Furthermore, mice with reduced NR1 subunits of the NMDA receptors display behavioral deficits, which are characteristic of schizophrenia (Mohn et al., 1999). Candidate risk genes for this disorder converge on the NMDAR expressing synapse, and a drug with a direct action on NMDA autoreceptors has shown clinical efficacy in schizophrenia treatment (Stone, 2009). Other reports showed that prenatal infections could cause hypofunction of glutamatergic system in the brain of adults as evidenced by hypersensitivity of these patients to psychotomimetic action of the uncompetitive NMDA receptor antagonist, ketamine.

Recently, we have proposed a modified model of schizophrenia based on repeated LPS injection in rats, that starts from 7th day of pregnancy and is continued every second day till delivery. In order to find out, whether this model is connected with functional dysregulation of glutamatergic system, the effect of the NMDA antagonist, MK801, on prepulse inhibition (PPI) and locomotor activity of adult offspring of prenatally LPS-exposed rats was measured. PPI is a phenomenon in which the motor response to a startling sensory stimulus is inhibited by a preceded sensory stimulus of a lower intensity, and its disturbance is thought to reflect attention deficits characteristic for schizophrenia. On the other hand, enhancement of MK-801-induced locomotor activity is regarded as positive symptom of this disorder. Since behavioral response to MK-801 depends on the interaction of glutamatergic and aminergic neuronal systems, additionally, the levels of dopamine (DA), noradrenaline (NA) and serotonin (5-HT) and their metabolites in the brains of prenatally LPS-treated rats were evaluated.

2. Materials and method

2.1. Experimental animals

Female and male Wistar rats (200 g) were obtained from Charles River Laboratories, Inc. (Germany) and were housed under standard experimental conditions (constant temperature of 22 + 2 °C) with an artificial 12-h light/dark cycle (the light on from 7:00 a.m. to 7:00 p.m.). All subjects used in the experiment were born in the experimental facilities of Institute of Pharmacology, Polish Academy of Sciences (Cracow, Poland). Dams and pups were housed in standard cages with wooden bedding and received food and water *ad libitum*.

2.2. Prenatal administration of LPS

Vaginal smears were taken daily from nulliparous females and, when found to be in oestrus, females were paired with males. Pregnancy was confirmed the next morning by the presence the vaginal smear (day 0 of pregnancy). Each pregnant female was housed individually. LPS from *Escherichia coli* (Sigma, L-3755, Serotype 026:B6) was administered subcutaneously to pregnant rats (n = 12) at a dose of 1 mg/kg every second day from the day 7 of pregnancy. LPS was dissolved in 0.9% NaCl and injected in a volume of 1.5 ml/kg between 9:00 and 10:00. The control group consisted of pregnant rats (n = 10) submitted to the same treatment schedule that were injected with an equivalent volume of 0.9% NaCl. After delivery the dams were allowed to rear their offspring until weaning. Five-day-old offspring were sexed and counted. No differences in the litter number and size were observed between LPS- and saline-treated rats. At the age of 21 days the offspring were weaned and caged in groups of six animals of the same sex and prenatal treatment. All experiments were performed on adult 90-day-old rats. The experimental protocols were approved by the Committee for Laboratory Animal Welfare and Ethics of the Institute of Pharmacology Polish Academy of Sciences, Cracow and met the criteria of the International Council for Laboratory Animals and Guide for the Care and Use of Laboratory Animals.

2.3. MK-801 administration

MK-801 hydrochloride (Sigma, St Louis, MO, USA) was dissolved in the 0.9% NaCl and administered intraperitoneally (i.p.) at the dose of 0.4 or 0.1 mg/kg in a volume of 2 ml/kg.

2.4. Sensorimotor gating

The startle apparatus (SR-LAB, San Diego Instruments, San Diego, CA) consisted of 12 individual soundproof ventilated chambers containing a non-restrictive Plexiglas cylinder (inner diameter = 9 cm) A high-frequency loudspeaker inside each chamber produced both a continuous background noise of 65 dB and the various acoustic stimuli. Movement of the Plexiglas cylinder caused by startle response of the animal was transduced into analogue signals by a piezoelectric unit attached to the platform. These signals were then digitized and stored by a computer. One hundred readings were taken at 1 ms intervals, starting at the stimulus onset, and the average amplitude was used to determine the average amplitude of startle reflex. The cage has been individually calibrated by the external sensor, in order to display a similar readout of the reference stimulus.

After habituation (5 min, background white noise, 65 dB), four types of acoustic stimuli were used in random order: acoustic stimulus alone–pulse of broad-band noise [intensity: 120 dB, duration: 40 ms, (P)] or the acoustic stimulus as aforementioned preceded by a acoustic prepulse and again a pulse of broad-band noise, [intensity: 71, 74, and 77 dB], duration: 20 ms; (PP)] applied 100 ms before the stimulus (P). During each experimental session, 20 trials of each type were presented with an interstimulus interval of 20 s. The amplitudes were averaged for each individual animal, separately for both types of trials [stimulus alone (P) or stimulus preceded by the prepulse (PP)]. The degree of prepulse inhibition was shown as a percentage of inhibition (% PPI) calculated as follows: ([(P-PP)/P] × 100). The procedure has been described previously by Wędzony et al. (2000, 2008). The injection of MK-801 for sensorimotor gating measurements was performed 30 min before the PPI test. The number of female and male rats in each group (vehicle; MK-801 0.4 mg/kg; MK-801 0.1 mg/kg) of control or prenatally LPS treated offspring was six to eight.

2.5. Measurement of locomotor activity

The locomotor activity of rats was recorded individually for each animal in Opto-Varimex cages (Columbus Instruments, OH), linked on line to an IBM-PC compatible computer. Each cage (43/44 cm) was equipped with 15 infrared emitters, located on the x and y axes, and an equivalent amount of receivers on the opposite walls of the cage. The locomotor activity of rats was analyzed using Auto-Track software (Columbus Instruments). The locomotor activity was defined as a trespass of three consecutive photo-beams, while other movements (e.g., repeated interruption of the same photo-beams) were regarded as non-locomotor movements (presumably movement of the body only). The locomotor activity is expressed as a distance travelled by animals in respective time intervals (10 or 60 min). The impact of LPS pretreatment in female and male offspring on locomotor hyperactivity induced by MK-801 (0.1 and 0.4 mg/kg, i.p) was analyzed in four groups of animals (six to eight animals per group); two were pretreated with LPS and two were treated with saline in the respective period, in a session lasting 150 min. Before recording, the animals were habituated to the Opto-Varimex cages for 30 min (data not shown). After that period, they were injected with vehicle (0.9% NaCl) and their locomotor activity was recorded for 60 min at 10 min intervals. At the end of that session, the rats were injected with MK-801 at the dose 0.1 mg/kg or 0.4 mg/kg and again their locomotor activity was registered for 60 min at 10 min intervals. Rats were used only once for locomotor activity test. All the data are given as the average distance travelled within 10 min intervals (time course) or

in a cumulative manner (60 min) ±SEM. The procedure has been described previously by Wędzony et al. (2000, 2008).

2.6. Measurement of tissue level of monoamines and their metabolites

After experiment was completed rats were decapitated, their brains were dissected out and brain regions (striatum and frontal cortex) were separated on ice. Tissue samples were weighted and homogenized in ice-cold 0.1 M perchloric acid. Then, homogenates were centrifuged at 10,000×*g*, supernatants were filtered through membrane filters (0.1 μm pore size) and were injected into HPLC system for determination of tissue level of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxytyramine (3-MT), as well as 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and noradrenaline (NA). Chromatography was performed using a Dionex P580 pump (USA), an LC-4C amperometric detector with a cross-flow detector cell (BAS, IN, USA) and BDS-Hypersil C18 analytical column (3×100 mm, a 3 μm, Thermo Electron Corp., UK). The mobile phase was composed of 0.05 M potassium dihydrogen phosphate (adjusted to pH=3.5 with ortho-phosphoric acid), 0.5 mM EDTA, 80 mg/l 1-octanesulfonic acid sodium salt, and a 4% methanol. The flow rate was 0.5 ml/min, and the applied potential of a 3 mm glassy carbon electrode was +600 mV with a sensitivity of 5 nA/V. The chromatographic data were processed by Chromax 2005 (Pol-Lab, Warszawa, Poland) software run on a personal computer.

2.7. Statistics

Results are presented as the group means±standard error of the mean (SEM). The data were analyzed by two-way analysis of variance for repeated measurements (ANOVA, MK-801 and LPS treatments were regarded as independent variables; amplitude of startle reflex evoked by acoustic tone alone or acoustic tone preceded by acoustic prepulse as a within factor for prepulse-induced inhibition of startle reflex) followed by the Duncan's post hoc test. Data on the percent of inhibition was evaluated by two-way ANOVA followed by the Duncan's post hoc test. Statistical evaluation utilized the Statistica program.

3. Results

3.1. The impact of MK801 on the amplitude of acoustic startle of LPS-pretreated male and female offspring

Analysis of startle response following a single systemic injection of MK-801 in a dose 0.1 and 0.4 mg/kg (two-way ANOVA for repeated measures; LPS pretreatment and two doses of MK-801 as independent factors, type of acoustic stimuli as a repeated measure) in male and female rats revealed significant overall effects of the prenatal LPS and MK-801 treatment and a significant effect of the type of acoustic stimuli (pulse alone or pulse preceded by prepulses). In both sexes a significant interaction between LPS×MK-801 was found. The significant interactions of type of acoustic stimuli×LPS, type of acoustic stimuli×MK-801 and type of acoustic stimuli×LPS×MK-801 were ascertained only in male rats (Table 1).

Post hoc comparisons for both sexes confirmed the previous observation that compared to VEH, the LPS-pretreated rats exhibited an increased basal startle amplitude evoked by the pulse alone (Fig. 1A, B). MK-801 in a dose 0.1 mg/kg enhanced the magnitude of startle amplitude of LPS-pretreated males and females, whereas no effect in control (VEH) groups was observed. Furthermore, it was noted that the prepulse-induced attenuation of startle amplitude evoked by tone, the trait feature observed in normal rats, was not affected by the low dose of MK-801 in male and female controls. Concurrently, MK-801 in a dose 0.4 mg/kg augmented the startle amplitude reciprocally in VEH and LPS-pretreated groups of both sexes. The magnitude of MK-801-induced increase in the amplitude was greater in LPS-pretreated rats than in controls. The high dose of MK-801 diminished the ability of prepulses to reduce startle amplitude evoked by acoustic tone observed in control rats (Fig. 1A, B).

Table 1
Effects of MK-801 (0.1 and 0.4 mg/kg) on the amplitude of acoustic startle of LPS-pretreated rats.

	Male	Female
LPS pretreatment	$F_{(1,42)}=3797$; $p<0.001$	$F_{(1,42)}=387$; $p<0.001$
MK-801 injection	$F_{(2,42)}=673$; $p<0.001$	$F_{(2,42)}=455$; $p<0.001$
LPS pretreatment × MK-801 injection	$F_{(2,42)}=123$; $p<0.001$	$F_{(2,42)}=13$; $p<0.001$
Type of acoustic stimuli	$F_{(3,126)}=80$; $p<0.001$	$F_{(3,126)}=13$; $p<0.001$
Type of acoustic stimuli × LPS pretreatment	$F_{(3,126)}=5$; $p<0.01$	$F_{(3,126)}=0.6$; $p>0.05$ ns
Type of acoustic stimuli × MK-801 injection	$F_{(6,126)}=5$; $p<0.001$	$F_{(6,126)}=1.3$; $p>0.05$ ns
Type of acoustic stimuli × LPS pretreatment × MK-801 injection	$F_{(6,126)}=3.4$; $p<0.01$	$F_{(6,126)}=0.4$; $p>0.05$ ns

3.2. The impact of MK-801 on the PPI of LPS-pretreated male and female offspring

Administration of MK-801 resulted in a dose-dependent and intensity-dependent disruption of PPI. Two-way repeated measures ANOVA (LPS and MK-801 as between-subject factors, prepulse intensity as repeated measure) demonstrated the significant effect of prenatal treatment with LPS and significant effect of a single injection of MK-801 (0.1 and 0.4 mg/kg) and significant impact of prepulse intensity on PPI in adult male and female rats. There was a significant interaction of LPS×MK-801, prepulse intensity×LPS and prepulse intensity×MK-801 but no significant interaction of prepulse intensity×LPS×MK-801 in both male and female offspring (Table 2).

Post hoc tests revealed that prenatal LPS pretreatment resulted in long-lasting disruptions of PPI in both male and female offspring (Fig. 2A, B). Moreover, the PPI was increased in the presence of greater prepulse intensities over all experimental groups.

Further analysis demonstrated that acute administration of the low dose of MK-801 (0.1 mg/kg) affected PPI only in LPS-pretreated groups. It was found that the low dose of MK-801 augmented the LPS-induced PPI deficit. Namely, the LPS-pretreated rats which received a single MK-801 injection (0.1 mg/kg) exhibited a greater reduction in PPI compared to LPS rats injected with saline. In VEH group, no effect of the low dose of MK-801 on PPI was observed. The high dose of MK-801 (0.4 mg/kg) produced a significant reduction of PPI in VEH- and LPS-pretreated animals, with the more robust effect being observed in female rats (Fig. 2A, B).

3.3. Impact of MK-801 on locomotor activity of LPS-pretreated male and female offspring

Examination of locomotor activity following a systemic injection of MK-801 in a dose 0.1 mg/kg (repeated measures ANOVA; LPS pretreatment and MK-801 injection as factors, consecutive time intervals as repeated measure) in male and female rats demonstrated significant overall effects of prenatal LPS and MK-801 in lower dose and a significant effect of the time intervals. In both sexes a significant interaction of LPS×MK-801 was found. Following the low dose of MK-801, no significant interaction of type of time interval×LPS×MK-801 was ascertained in both sexes (Table 3). Similar significant effects of both factors/treatments have been observed when data has been evaluated in a cumulative manner (see Fig. 3A, B, inset). Post hoc tests revealed that LPS-pretreated offspring of both sexes exhibited slightly

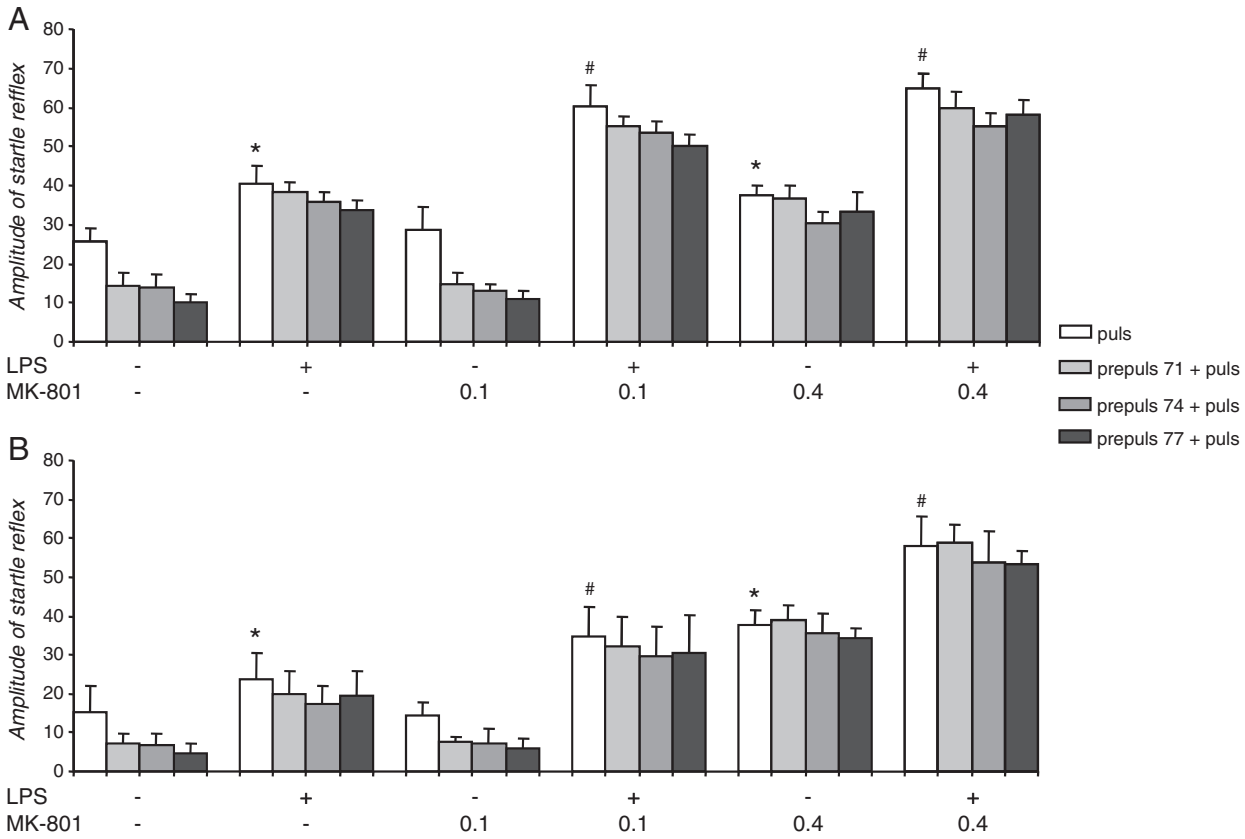

Fig. 1. The impact of MK801 (0.1 and 0.4 mg/kg) on the amplitude of startle reflex evoked by acoustic pulse (P) alone and pulse preceded by prepulse (PP) in adult male (A) and female (B) rats prenatally treated with LPS. The intensity of P was 120 dB, PP were applied at three different intensities: 71, 74 and 77 dB. * vs. VEH group. # vs. LPS + VEH. Repeated measures ANOVA and Duncan's post hoc test, $p<0.05$. All data are given as mean values ± SEM. N = 6–8.

enhanced basal spontaneous locomotor activity in comparison to respective controls. MK-801 in a dose 0.1 mg/kg failed to alter the locomotor activity of VEH group. In contrast, LPS-pretreated male and female rats displayed an increased locomotor activity in response to the low dose of MK-801, lasting 10–40 min after MK-801 administration (Fig. 3A, B).

Subsequent inspection of locomotor activity evoked by the higher dose of MK-801 (0.4 mg/kg) revealed significant main effects (LPS pretreatment and acute MK-801) and a significant effect of the time intervals in both sexes (repeated measures ANOVA). However, there were no significant LPS × MK-801 interactions in males and females indicating that MK-801 given in a dose 0.4 mg/kg resulted in a significant increase of the activity in both groups (Table 4). Similar significant effects of both factors/treatments and no significant interaction have been obtained in two-way ANOVA analysis of cumulative data. As shown in Fig. 4A and B, MK-801 given in a dose 0.4 mg/kg evoked a massive enhancement of locomotor activity in VEH- and LPS-pretreated groups, reaching maximal values 20–40 min after MK-801 injection. Maternal LPS treatment failed to affect the enhancement of locomotor activity evoked by the high dose of MK-801.

Table 2
Effects of MK-801 (0.1 and 0.4 mg/kg) on the PPI of LPS-pretreated rats.

	Male	Female
LPS pretreatment	$F_{(1,42)}=205$; $p<0.001$	$F_{(1,42)}=175$; $p<0.001$
MK-801 injection	$F_{(2,42)}=140$; $p<0.001$	$F_{(2,42)}=191$; $p<0.001$
LPS × MK-801 interaction	$F_{(2,42)}=22$; $p<0.001$	$F_{(2,42)}=21$; $p<0.001$
Prepulse intensity	$F_{(2,84)}=67$; $p<0.001$	$F_{(2,84)}=58$; $p<0.001$
Prepulse intensity × LPS interaction	$F_{(2,84)}=3$; $p<0.05$	$F_{(2,84)}=6.7$; $p<0.05$
Prepulse intensity × MK-801 interaction	$F_{(4,84)}=3$; $p<0.05$	$F_{(4,84)}=8.5$; $p<0.001$
Prepulse intensity × LPS × MK-801 interaction	$F_{(4,84)}=0.3$; $p>0.05$ ns	$F_{(4,84)}=0.4$; $p>0.05$ ns

3.4. Impact of maternal LPS treatment on monoamines in the striatum and frontal cortex of male and female offspring prenatally exposed to LPS

The levels of DA, 5-HT, NA and their metabolites were measured in male and female offspring of dams exposed to LPS or saline in pregnancy (Table 5). In the striatum, post hoc analysis revealed that the tissue levels of DA were significantly higher in LPS-pretreated female ($F_{(1,12)}=7.71$; $p<0.01$) but not in male ($F_{(1,11)}=4.53$; $p>0.05$; ns) offspring. Striatal levels of DOPAC were higher in both LPS-pretreated female ($F_{(1,10)}=6.34$; $p<0.05$) and male ($F_{(1,11)}=7.61$; $p<0.05$) offspring. There were no statistically significant changes in the level of the extraneuronal metabolite of DA, 3-MT, and the level of 5-HT and its metabolite, 5-HIAA between LPS-pretreated and control animals in the striatum.

In the frontal cortex, a post hoc analysis revealed that the DA content was lower in LPS-pretreated females ($F_{(1,8)}=7.87$; $p<0.05$) and males ($F_{(1,11)}=5.80$; $p<0.05$). Similarly, a significant decrease in the DA metabolite, DOPAC was observed in LPS-pretreated females ($F_{(1,11)}=8.09$; $p<0.05$) and males ($F_{(1,11)}=8.93$; $p<0.05$). Moreover, LPS-pretreated females showed higher 5-HT ($F_{(1,11)}=14.22$; $p<0.01$) and its metabolite, 5-HIAA ($F_{(1,12)}=12.14$; $p<0.01$) levels in the frontal cortex, whereas in male offspring no changes in these parameters were observed. Additionally, no statistically significant changes in NA concentrations in the frontal cortex between LPA-pretreated and control animals were detected.

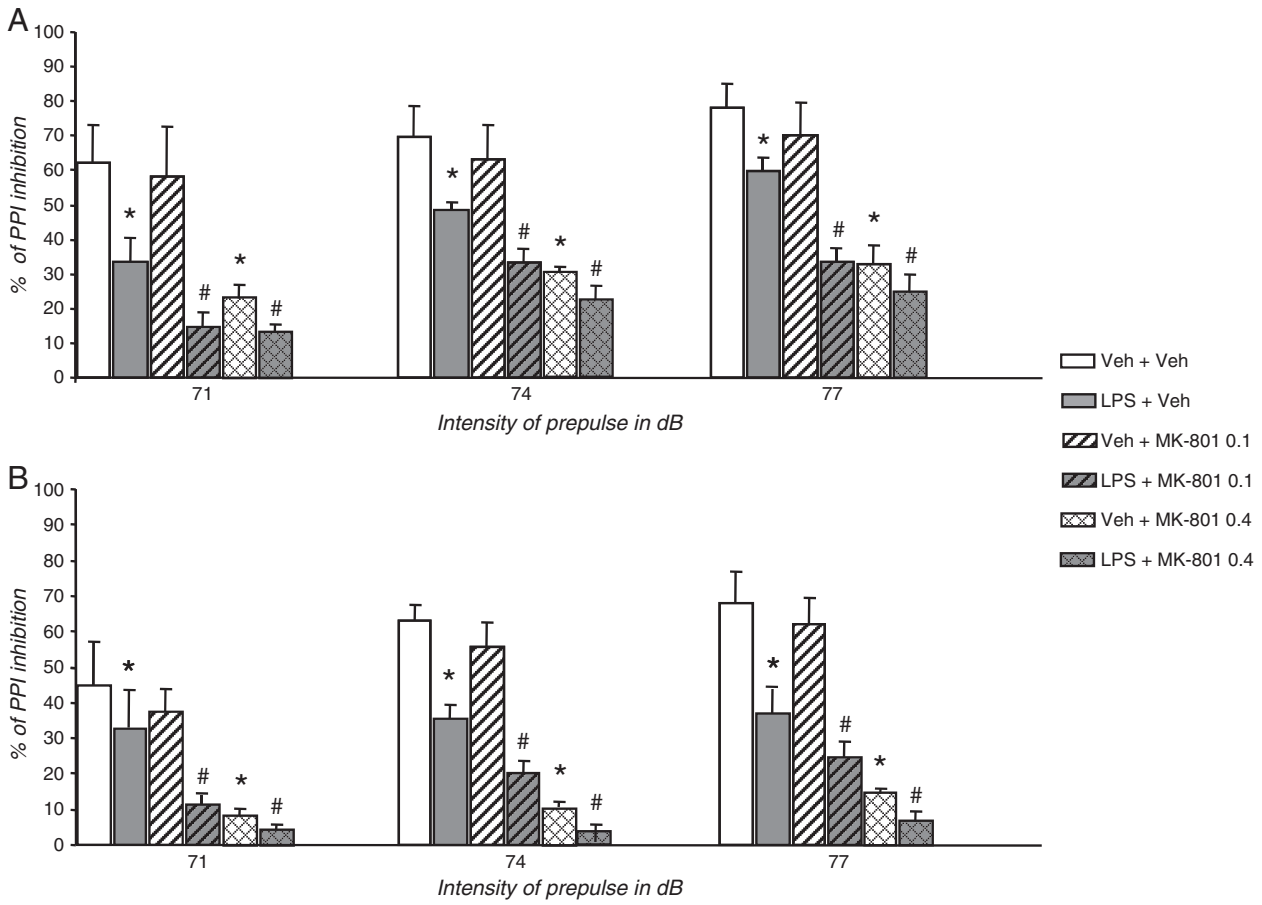

Fig. 2. The impact of MK801 (0.1 and 0.4 mg/kg) on prepulse inhibition of acoustic startle response (PPI) in adult male (A) and female (B) rats prenatally treated with LPS. The intensity of P was 120 dB, PP were applied at three different intensities: 71, 74 and 77 dB. PPI is expressed as a percentage of inhibition. * vs. VEH group. # vs. LPS + VEH. Repeated measures ANOVA and Duncan's post hoc test, $p<0.05$. All data are given as the mean values ± SEM. N = 6–8.

4. Discussion

The present data showed that LPS administration in the second and third week of pregnancy was sufficient to induce schizophrenia-like symptoms in adult rat offspring. Thus both male and female offspring of LPS-pretreated rats exhibited an increased basal startle amplitude evoked by pulse alone and a long-lasting disruption of PPI. These results closely resemble those obtained in other animal models based on prenatal immunoactivation by the bacterial endotoxin administered throughout the whole pregnancy period or in a significantly higher dose than that employed in our study (Romero et al., 2008; Borrell et al., 2002) and by viral mimic polyinosinic: polycytidylic acid (poly I:C) (Fortier et al., 2007). Another finding of this study was the demonstration that prenatal LPS led to persistent elevation of locomotor activity in adult offspring, the paradigm relevant to psychotic-like symptoms (Lipska and Weinberger, 2000), and this indicates, that the model reconstructs some clinical aspects of schizophrenia. It should be mentioned here that MK-801 is known to evoke locomotor hyperactivity, stereotypy and sniffing behavior as well as disruption of sensorimotor gating measured as a deficit in prepulse inhibition (PPI) of acoustic startle response (Koek et al., 1988; Wędzony et al., 1994, 2000).

In line with other studies, MK-801 in a high dose (0.4 mg/kg) increased the acoustic startle amplitude, reduced PPI, and enhanced locomotor activity in both control and LPS-pretreated animals, whereas the lower dose (0.1 mg/kg) was inactive in control rats. The prenatally LPS-treated rats showed hypersensitivity to MK-801 as evidenced not only by the enhancement of the effects of its high dose, but also by ability of the lower dose to change significantly all the aforementioned parameters. The abnormal behavioral response to MK-801 that we found in prenatally LPS-treated rats suggests changes especially in dopaminergic and glutamatergic systems since PPI is connected with an increased dopamine activity in the nucleus accumbens, whereas augmented locomotor activity is linked with an increase in this neurotransmitter content in the striatum. In fact, a current theory of

Table 3
Effects of MK-801 (0.1 mg/kg) on the locomotor activity of LPS-pretreated rats.

	Male	Female
Repeated measures ANOVA (time intervals as repeated measures)		
LPS pretreatment	$F_{(1,20)}=69$; $p<0.001$	$F_{(1,20)}=27$; $p<0.001$
MK-801 injection	$F_{(1,20)}=28$; $p<0.001$	$F_{(1,20)}=9$; $p<0.001$
LPS pretreatment × MK-801 injection	$F_{(1,20)}=23$; $p<0.001$	$F_{(1,20)}=4$; $p<0.001$
Time intervals	$F_{(5,100)}=36$; $p<0.001$	$F_{(5,100)}=6$; $p<0.001$
Time intervals × LPS pretreatment	$F_{(5,100)}=4.6$; $p<0.01$	$F_{(5,100)}=24.6$; $p<0.01$
Time intervals × MK-801 injection	$F_{(5,100)}=1.8$; $p>0.05$ ns	$F_{(5,100)}=2$; $p>0.05$ ns
Time intervals × LPS pretreatment × MK-801 injection	$F_{(5,100)}=1.2$; $p>0.05$ ns	$F_{(5,100)}=1$; $p>0.05$ ns
Two-way ANOVA (cumulative data)		
LPS pretreatment	$F_{(1,44)}=132$; $p<0.001$	$F_{(1,44)}=57$; $p<0.001$
MK-801 injection	$F_{(1,44)}=65$; $p<0.001$	$F_{(1,44)}=18$; $p<0.001$
LPS pretreatment × MK-801 injection	$F_{(1,44)}=52$; $p<0.001$	$F_{(1,44)}=7.7$; $p<0.001$

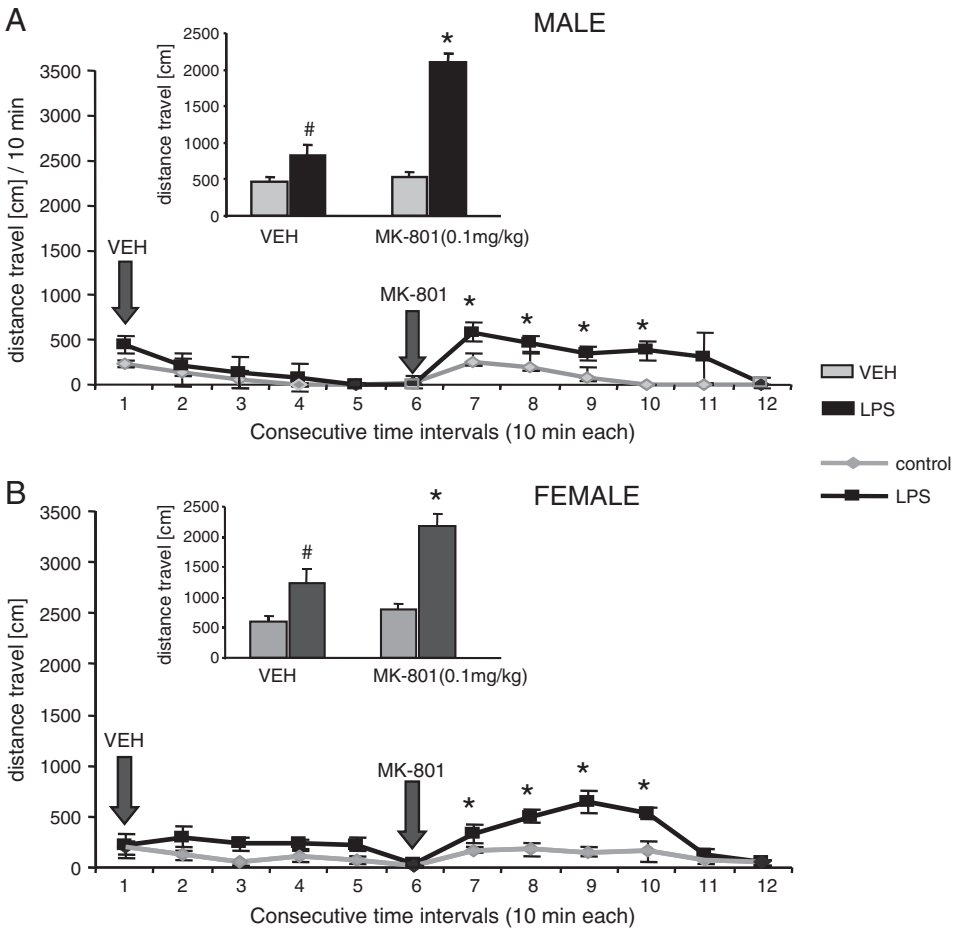

Fig. 3. The impact of MK801 (0.1 mg/kg) on locomotor activity of male (A) and female (B) adult rats prenatally treated with LPS. Locomotor activity (distance travelled) was measured (after habituation lasting 30 min, data not shown) for 60 min after saline (SAL) injection and then for 60 min after MK801 treatment. Insets show cumulative data for the entire sessions lasting 60 min. Arrows indicate the time of SAL and MK801 administration. * Significant differences in locomotor activity between LPS and VEH groups after a single injection of MK801 (0.1 mg/kg). Repeated measures ANOVA, $p<0.05$. Inset: # LPS vs. VEH group. * LPS + MK801(0.1 mg/kg) vs. LPS + VEH. Two-way ANOVA and Duncan's post hoc test, $p<0.05$. All data are given as mean values ± SEM. N = 6–8.

schizophrenia points to an imbalance in dopaminergic and glutamatergic systems, especially on glutamate and dopamine hypofunction in the frontal cortical areas and enhanced striatal dopamine neurotransmission (Carlsson et al., 1999; Paz et al., 2008; Tamminga, 1998). A disturbance in dopaminergic and glutamatergic systems may result from the brain immunoactivation during pregnancy. This statement is supported by the observations that infections or inflammatory processes in perinatal period induce long-lasting alterations in the central neurotransmitter systems, including dopaminergic ones (Kabiersch et al., 1998; Muller and Schwarz, 2006). These effects may be mediated via cytokines since prenatally administered LPS was reported to increase some cytokine levels (Romero et al., 2008).

Table 4
Effects of MK-801 (0.4 mg/kg) on the locomotor activity of LPS-pretreated rats.

	Male	Female
Repeated measures ANOVA (time intervals as repeated measures)		
LPS pretreatment	$F_{(1,20)}=69$; $p<0.001$	$F_{(1,20)}=27$; $p<0.001$
MK-801 injection	$F_{(1,20)}=335$; $p<0.001$	$F_{(1,20)}=78$; $p<0.001$
LPS pretreatment × MK-801 injection	$F_{(1,20)}=0.4$; $p>0.05$ ns	$F_{(1,20)}=0.2$; $p>0.05$ ns
Time intervals	$F_{(5,100)}=23$; $p<0.001$	$F_{(5,100)}=9$; $p<0.001$
Time intervals × LPS pretreatment	$F_{(5,100)}=2.8$; $p<0.05$	$F_{(5,100)}=0.6$; $p>0.05$ ns
Time intervals × MK-801 injection	$F_{(5,100)}=33$; $p<0.001$	$F_{(5,100)}=11$; $p<0.001$
Time intervals × LPS pretreatment × MK-801 injection	$F_{(5,100)}=1.2$; $p>0.05$ ns	$F_{(5,100)}=0.4$; $p>0.05$ ns
Two-way ANOVA (cumulative data)		
LPS pretreatment	$F_{(1,44)}=132$; $p<0.001$	$F_{(1,44)}=57$; $p<0.001$
MK-801 injection	$F_{(1,44)}=737$; $p<0.001$	$F_{(1,44)}=171$; $p<0.001$
LPS pretreatment × MK-801 injection	$F_{(1,44)}=0.8$; $p>0.05$ ns	$F_{(1,44)}=0.3$; $p>0.05$ ns

Impact of maternal LPS treatment on monoamines in the striatum and frontal cortex of male and female offspring prenatally exposed to LPS.

A question arises what mechanism might be responsible for the hypersensitivity of prenatally LPS-treated animals to an NMDA receptor antagonist. It is likely that the activation of the immune system in prenatal period of neurodevelopment leads to hypoactivity of glutamatergic system. This assumption is supported by the observation that the inhibition of glutamatergic system activity by noncompetitive NMDA receptor antagonists, like phencyclidine, ketamine and dizocilpine (MK-801) produces strong psychotomimetic effects in several behavioral models. Interestingly, a prolonged blockade of NMDA receptor in perinatal period produces the behavioral symptoms in animals resembling those observed in LPS-pretreated rats (Wędzony et al., 2008; Wang et al., 2001; Becker et al., 2003; Facchinetti et al., 1994).

Furthermore, mice with reduced NMDA receptor expression display behaviors related to schizophrenia (Mohn et al., 1999). It has also been postulated that the imbalance between type 1 and type 2 immune response described in some schizophrenic patients leads to an increase in brain kynurenic acid level, which as an endogenous NMDA receptor antagonist may reduce glutamate system activity (Müller et al., 1991;

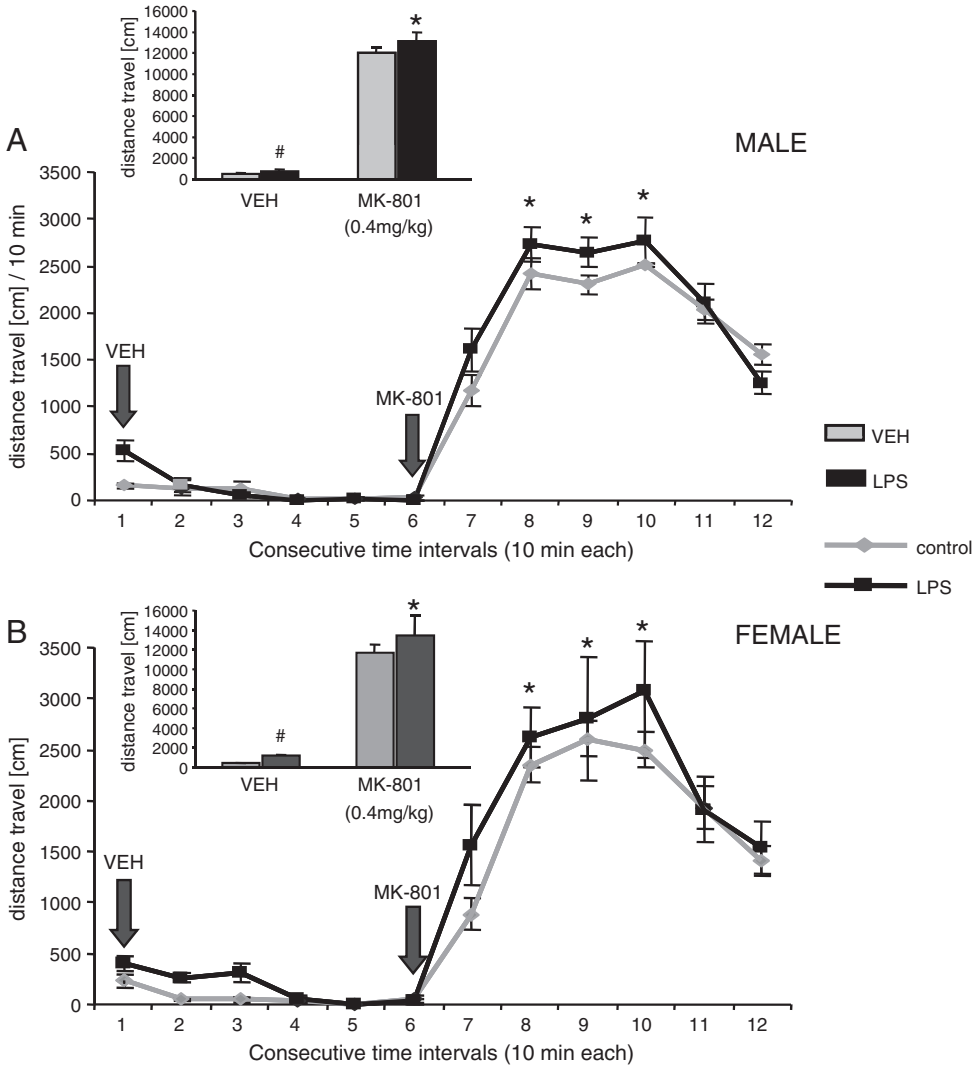

Fig. 4. The impact of MK801 (0.4 mg/kg) on locomotor activity of male (A) and female (B) adult rats prenatally treated with LPS. Locomotor activity (distance traveled) was measured (after habituation lasting 30 min, data not shown) for 60 min after saline (SAL) injection and then for 60 min after MK801 treatment. Insets show cumulative data for the entire session lasting 60 min. Arrows indicate the time of SAL and MK801 administration. MK801 (0.4 mg/kg) enhanced the locomotor activity of LPS and VEH pretreated rats. * Significant overall effect of MK801 (0.4 mg/kg).Two-way ANOVA, $p<0.05$.Inset:# LPS vs. VEH group.* LPS + MK801 (0.1 mg/kg) vs. LPS + sal.Two-way ANOVA and Duncan's post hoc test, $p<0.05$.All data are given as mean values ± SEM. N = 6–8.

Table 5
Tissue concentration of DA, DOPAC, 3-MT, NA, 5-HT, 5-HIAA (pg/mg weight tissue) in striatum and prefrontal cortex in female and male control and LPS-pretreated rats offspring.

		Female		Male	
		Control	LPS-pretreated	Control	LPS-pretreated
Striatum	DA	7915.8 ± 34.1	9331.3 ± 43.9*	8492.8 ± 80.0	9132.6 ± 79.3
	DOPAC	1049.0 ± 54.3	1313.7 ± 39.0*	1229.2 ± 23.3	1409.3 ± 44.1*
	3-MT	333.2 ± 32.0	360.0 ± 21.6	333.4 ± 15.5	423.4 ± 13.9
	5-HT	521.2 ± 93.2	484.0 ± 47.7	519.7 ± 36.6	494.7 ± 41.9
	5-HIAA	611.0 ± 80.6	559.0 ± 53.2	667.5 ± 19.9	659.5 ± 64.3
Frontal cortex	DA	884.3 ± 38.6	644.3 ± 31.2*	875.7 ± 26.7	585.6 ± 22.3*
	DOPAC	134.0 ± 18.4	107.4 ± 15.3*	141.0 ± 13.4	100.3 ± 20.8*
	3-MT	18.7 ± 5.0	14.7 ± 3.9	13.2 ± 2.7	15.4 ± 10.0
	5-HT	151.8 ± 21.8	277.3 ± 26.6*	270.8 ± 20.7	265.7 ± 20.4
	5-HIAA	81.5 ± 8.0	146.6 ± 20.2*	150.7 ± 31.3	158.9 ± 30.0
	NA	223.3 ± 40.0	222.1 ± 19.2	230.2 ± 22.7	229.6 ± 30.0

Abbreviations:
DA — dopamine.
NA — noradrenaline.
LPS — lipopolysaccharide.
DOPAC — dihydroxyphenylacetic acid.
5-HT — serotonine.
3 MT — 3-methoxytyramine.
5 -HIAA — 5-hydroxyindolacetic acid.
Results are expressed as the mean + SEM (n = 5–8 animals per group). * $p<0.01$ vs. control rats (ANOVA followed by Duncan's test).

Muller and Schwarz, 2006; Sumiyoshi et al., 2004). It is then not unlikely that prenatal LPS administration may cause hypoactivity of glutamatergic neurons via a disturbance in pro- and anti-inflammatory cytokine production (Borell et al., 2002). The hyporeactivity of NMDA receptors may in turn result in disinhibition of nigro-striatal dopaminergic neurons (Imperato et al., 1990; Miller and Abercrombie, 1996). In accordance with this suggestion, we observed the elevated level of dopamine in the striatum of LPS-pretreated rats, however, this effect reached statistical significance only in females. Similarly, Romero et al., (2008) reported a non-significant tendency to increase striatal DA level in 170-day-old male rats following prenatal LPS treatment. The increased level of DA in the striatum of females may contribute to the enhanced locomotor activity. Importantly, in males and females, a significant increase in the striatal DOPAC content was detected, which indicates an augmentation of dopamine turnover, and correlates well with the enhanced locomotor activity in the LPS-pretreated animals. It should be also stressed that some studies do not support the hypothesis that dopamine is involved in the MK-801-induced hyperactivity. For instance, MK-801 robustly increases locomotion in the absence of an increased dopamine overflow in rats (Druhan et al 1996), and after dopamine depletion in mice (Carlsson and Carlsson, 1989; Carlsson and Svensson, 1990).

Furthermore, it has been postulated that the higher activity of striatal dopaminergic neurons may be connected with an attenuated prefrontal dopamine transmission (Akil et al., 2003). In line with the aforementioned information, in our neurodevelopmental model of schizophrenia a significant decrease in dopamine and its intracellular metabolite, DOPAC in the frontal cortex was found both in male and female rats. The reduced dopamine neurotransmission in the cerebral cortex has been described in schizophrenic patients (Akil et al., 2000; Wędzony et al., 2005) and in animal models of this disease (Wędzony et al., 2005; Dall'Olio et al., 1994). The diminished cortical dopaminergic system activity is ascribed to the metabolic hypofrontality, which is typical of cognitive deficits and negative symptoms of schizophrenia, and in animal models it is reflected by PPI deficit (Schneider and Koch, 2005).

In contrast to dopamine system, no changes in the cortical level of noradrenaline were found, which suggests that LPS treatment in prenatal period affects only specific populations of catecholaminergic neurons. Notably, the most robust change in the brain of LPS-pretreated rats was an increase in 5-HT and its metabolite 5-HIAA level in the frontal cortex of female rats. Although the role of serotonin in pathophysiology of schizophrenia is still unclear, recent data provided evidence of increased serotonergic neurotransmission in schizophrenic patients (Juckel et al., 2008; Gudlowski et al., 2009). Moreover, the most consistent early findings showed the increased level of serotonin in discrete brain structures including prefrontal cortex. Since changes in the levels of 5-HT and 5-HIAA were detected only in females, it is likely that they result from alterations in the levels of some hormones which regulate serotonergic transmission, e.g. prolactin and growth hormone (Kaplan and Sadock, 1995).

Collectively, the present study has shown that the immune system activation in prenatal period leads to persistent behavioral hypersensitivity to psychotomimetic action of a non-competetive NMDA receptor antagonist. These behavioral changes were accompanied by the enhanced striatal and the decreased cortical dopaminergic system activity in both sexes, whereas augmentation of serotonin neurotransmission in the frontal cortex was found in females only. These results emphasize the role of glutamatergic–dopaminergic–serotoninergic system interaction in the immune-dependent mechanism of etiopathogenesis of schizophrenia.

Acknowledgements

The authors wish to thank Ms. B. Korzeniak for her skilful technical assistance.

References

Akil M, Edgar ChL, Pierri JN, Casali S, Lewis DA. Decreased density of thyrosine hydroxylase-immunoreactive axons in the enthorinal cortex of schizophrenic subjects. Biol Psychiatry 2000;47:361–70.
Akil M, Kolachana BS, Rothmond DA, Hyde TM, Weinberger DR, Kleinman JE. Catechol-O-methyltransferase genotype and dopamine regulation in the human brain. J Neurosci 2003;23:2008–13.
Baharnoori M, Brake WG, Srivastava LK. Prenatal immune challenge induces developmental changes in the morphology of pyramidal neurons of the prefrontal cortex and hippocampus in rats. Schizophr Res 2009;107:99-109.
Becker A, Peters B, Schroeder H, Mann T, Huether G, Grecksch G. Ketamine-induced changes in rat behaviour: a possible animal model of schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:687–700.
Borrell J, Vela JM, Arévalo-Martin A, Molina-Holgado E, Guaza C. Prenatal immune challenge disrupts sensorimotor gating in adult rats. Implications for the etiopathogenesis of schizophrenia. Neuropsychopharmacology 2002;26:204–15.
Brown AS, Begg MD, Gravenstein S, Schaefer CA, Wyatt RJ, Bresnahan M, et al. Serologic evidence of prenatal influenza in the etiology of schizophrenia. Arch Gen Psychiatry 2004;61:774–80.
Cai Z, Pan ZL, Pang Y, Evans OB, Rhodes PG. Cytokine induction in fetal rat brains and brain injury in neonatal rats after maternal lipopolysaccharide administration. Pediatr Res 2000;47:64–72.
Carlsson M, Carlsson A. Dramatic synergism between MK-801 and clonidine with respect to locomotor stimulatory effect in monoamine-depleted mice. J Neural Transm 1989;77:65–71.
Carlsson M, Svensson A. The non-competitive NMDA antagonists MK-801 and PCP, as well as the competitive NMDA antagonist SDZ EAA494 (D-CPPene), interact synergistically with clonidine to promote locomotion in monoamine-depleted mice. Life Sci 1990;47:1729–36.
Carlsson A, Waters N, Carlsson ML. Neurotransmitter interactions in schizophrenia — therapeutic implications. Biol Psychiatry 1999;46:1388–95.
Dall'Olio R, Facchinetti F, Contestabile A, Gandolfi O. Chronic neonatal blockade of N-methyl-D-aspartate receptor by CGP 39551 increases dopaminergic function in adult rat. Neuroscience 1994;63:451–5.
Druhan JP, Rajabi H, Stewart J. MK-801 increases locomotor activity without elevating extracellular dopamine levels in the nucleus accumbens. Synapse 1996;24:135–46.
Facchinetti F, Dall'Olio R, Ciani E, Sparapani M, Virgili M, Contestabile A. Long-lasting effects of chronic neonatal blockade of N-methyl-D-aspartate receptor through the competitive antagonist CGP 39551 in rats. Neuroscience 1994;60:343–53.
Fortier ME, Luheshi GN, Boksa P. Effects of prenatal infection on prepulse inhibition in the rat depend on the nature of the infectious agent and the stage of pregnancy. Behav Brain Res 2007;181:270–7.
Gudlowski Y, Ozgürdal S, Witthaus H, Gallinat J, Hauser M, Winter C, et al. Serotonergic dysfunction in the prodromal, first-episode and chronic course of schizophrenia as assessed by the loudness dependence of auditory evoked activity. Schizophr Res 2009;109:141–7.
Imperato A, Scrocco MG, Bacchi S, Angelucci L. NMDA receptors and in vivo dopamine release in the nucleus accumbens and caudatus. Eur J Pharmacol 1990;187:555–6.
Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 1991;148:1301–8.
Juckel G, Gudlowski Y, Müller D, Ozgürdal S, Brüne M, Gallinat J, et al. Loudness dependence of the auditory evoked N1/P2 component as an indicator of serotonergic dysfunction in patients with schizophrenia — a replication study. Psychiatry Res 2008;158:79–82.
Kabiersch A, Furukawa H, del Rey A, Besedovsky HO. Administration of interleukin-1 at birth affects dopaminergic neurons in adult mice. Ann NY Acad Sci 1998;840:123–7.
Koek W, Woods JH, Winger GD. MK-801, a proposed noncompetitive antagonist of excitatory amino acid neurotransmission, produces phencyclidine-like behavioral effects in pigeons, rats and rhesus monkeys. J Pharmacol Exp Ther 1988;245:969–74.
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.
Lipska BK, Weinberger DR. To model a psychiatric disorder in animals: schizophrenia as a reality test. Neuropsychopharmacolo 2000;23:223–39.
Mednick SA, Machon RA, Huttunen MO, Bonett D. Adult schizophrenia following prenatal exposure to an influenza epidemic. Arch Gen Psychiatry 1988;45:189–92.
Miller DW, Abercrombie ED. Effects of MK-801 on spontaneous and amphetamine-stimulated dopamine release in striatum measured with in vivo microdialysis in awake rats. Brain Res Bull 1996;40:57–62.
Mohn AR, Gainetdinov RR, Caron MG, Koller BH. Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. Cell 1999;98:427–36.
Müller N, Ackenheil M, Hofschuster E, Mempel W, Eckstein R. Cellular immunity in schizophrenic patients before and during neuroleptic treatment. Psychiatry Res 1991;37:147–60.
Muller N, Schwarz M. Schizophrenia as an inflammation-mediated dysbalance of glutamatergic neurotransmission. Neurotox Res 2006;10:131–48.
Nawa H, Takahashi M, Patterson PH. Cytokine and growth factor involvement in schizophrenia — support for the developmental model. Mol Psychiatry 2000;5:594–603.
O'Callaghan E, Sham PC, Takei N, Murray G, Glover G, Hare EH, et al. The relationship of schizophrenic births to 16 infectious diseases. Br J Psychiatry 1994;165:353–6.

Pauli S, Linthorst AC, Reul JM. Tumour necrosis factor-alpha and interleukin-2 differentially affect hippocampal serotonergic neurotransmission, behavioural activity, body temperature and hypothalamic–pituitary–adrenocortical axis activity in the rat. Eur J Neurosci 1998;10:868–78.

Paz RD, Tardito S, Atzori M, Tseng KY. Glutamatergic dysfunction in schizophrenia: from basic neuroscience to clinical psychopharmacology. Eur Neuropsychopharm 2008;18: 773–86.

Pollmächer T, Schreiber W, Gudewill S, Vedder H, Fassbender K, Wiedemann K, et al. Influence of endotoxin on nocturnal sleep in humans. Am J Physiol 1993;264: R1077–83.

Romero E, Guaza C, Castellano B, Borrell J. Ontogeny of sensorimotor gating and immune impairment induced by prenatal immune challenge in rats: implications for the etiopathology of schizophrenia. Mol Psychiatry 2008:1-12.

Schneider M, Koch M. Behavioral and morphological alterations following neonatal excitotoxic lesions of the medial prefrontal cortex in rats. Exper Neurol 2005;195: 185–98.

Shi L, Fatemi SH, Sidwell RW, Patterson PH. Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. J Neurosci 2003;23:297–302.

Stone JM. Imaging the glutamate system in humans: relevance to drug discovery for schizophrenia. Curr Pharm Des 2009;15:2594–602.

Sumiyoshi T, Anil AE, Jin D, Jayathilake K, Lee M, Meltzer HY. Plasma glycine and serine levels in schizophrenia compared to normal controls and major depression: relation to negative symptoms. Int J Neuropsychopharmacol 2004;7:1–8.

Tamminga CA. Schizophrenia and glutamatergic transmission. Crit Rev Neurobiol 1998;12:21–36.

Tohmi M, Tsuda N, Watanabe Y, Kakita A, Nawa H. Perinatal inflammatory cytokine challenge results in distinct neurobehavioral alterations in rats: implication in psychiatric disorders of developmental origin. Neurosci Res 2004;50:67–75.

Wang C, McInnis J, Ross-Sanchez M, Shinnick-Gallagher P, Wiley JL, Johnson KM. Long-term behavioral and neurodegenerative effects of perinatal phencyclidine administration: implications for schizophrenia. Neuroscience 2001;107:535–50.

Watanabe Y, Hashimoto S, Kakita A, Takahashi H, Ko J, Mizuno M, et al. Neonatal impact of leukemia inhibitory factor on neurobehavioral development in rats. Neurosci Res 2004;48:345–53.

Wędzony K, Gołembiowska K, Zazula M. Differential effects of CGP 37849 and MK-801, competitive and noncompetitive NMDA antagonists, with respect to the modulation of sensorimotor gating and dopamine outflow in the prefrontal cortex of rats. Naunyn Schmiedebergs Arch Pharmacol 1994;350:555–62.

Wędzony K, Maćkowiak M, Zajączkowski W, Fijał K, Chocyk A, Czyrak A. WAY 100135, an antagonist of 5-HT1A serotonin receptors, attenuates psychotomimetic effects of MK-801. Neuropsychopharmacology 2000;23:547–59.

Wędzony K, Fijał K, Chocyk A. Blockade of NMDA receptors in postnatal period decreased density of tyrosine hydroxylase immunoreactive axonal arbors in the medial prefrontal cortex of adult rats. J Physiol Pharmacol 2005;56:205–21.

Wędzony K, Fijał K, Maćkowiak M, Chocyk A, Zajączkowski W. Impact of postnatal blockade of N-methyl-D-aspartate receptors on rat behavior: a search for a new developmental model of schizophrenia. Neuroscience 2008;60:856–64.

Zuckerman L, Weiner I. Maternal immune activation leads to behavioral and pharmacological changes in the adult offspring. J Psychiatr Res 2005;39:311–23.