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Research report

Lipopolysaccharide exposure during late embryogenesis results in diminished locomotor activity and amphetamine response in females and spatial cognition impairment in males in adult, but not adolescent rat offspring

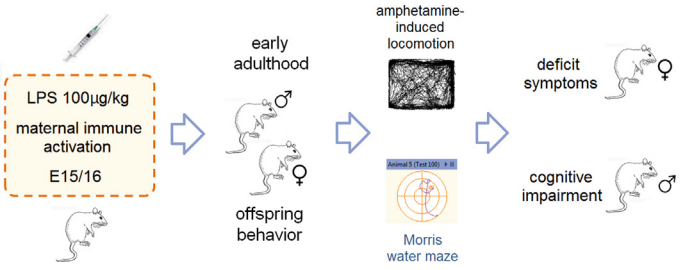
Bojan Batinić[a], Anja Santrač[b], Branka Divović[b], Tamara Timić[b], Tamara Stanković[b], Aleksandar Lj Obradović[a], Srđan Joksimović[b], Miroslav M. Savić[b,*]

[a] Department of Physiology, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11221 Belgrade, Serbia
[b] Department of Pharmacology, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11221 Belgrade, Serbia

HIGHLIGHTS

- Effects of LPS applied in late embryogenesis were tested at postnatal days 40 and 60.
- Male rats exhibited spatial learning and memory impairments only in early adulthood.
- Female rats displayed decreased reactivity to amphetamine only in early adulthood.
- Gender differences may mimic cognitive dysfunction and deficit symptoms, respectively.

GRAPHICAL ABSTRACT



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ABSTRACT

Numerous basic and epidemiological studies have connected prenatal maternal immune activation with the occurrence of schizophrenia and/or autism. Depending on subtle differences in protocols of the used animal model, a variety of behavioral abnormalities has been reported. This study investigated behavioral differences in Wistar rat offspring of both genders, exposed to the 100 μg/kg per day dose of lipopolysaccharide (LPS) in late embryogenesis (embryonic days 15 and 16), while tested at their adolescent and young adult age (postnatal days 40 and 60, respectively). Immune activation was confirmed by detecting high levels of TNF-α and IL-6 in dam blood withdrawn 2 h after the first dose of LPS. The animals were assessed in three consecutive trials of locomotor activity (novelty exploration, response to *i.p.* saline injection and challenge with 0.5 mg/kg amphetamine), Morris water maze and social interaction tests. Overt behavioral dysfunction was perceived in adult rats only, and these changes were gender-distinctive. When compared with control rats, LPS females displayed baseline hypolocomotion and a decreased reactivity to amphetamine, while LPS males exhibited spatial learning (acquisition trials) and memory (probe trial) impairments. Prenatal treatment did not affect the time spent in social interaction. As maternal exposure to LPS in late gestation resulted in behavioral changes in offspring in early adulthood, it may model schizophrenia-like, but not autism-like endophenotypes. However, lack of a potentiated response to amphetamine testified that this model could not mimic positive symptoms, but rather certain traits of cognitive dysfunction and deficit symptoms, in males and females, respectively.

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1. Introduction

Disturbances of in utero brain development are considered to underlie the emergence of various neurological and psychiatric disorders later in life. A number of epidemiological studies introduced maternal immune activation as an etiological factor in schizophrenia, indicating that prenatal exposure to bacterial or viral insults carries an increased risk of disorder development [1–3]. Similarly, etiology of autism, initially regarded as of primarily genetic origin, was remodeled in its bases and exposure to maternal immune activation was recognized as one of the risk factors [4,5]. Parkinson's and Alzheimer's disease, cerebral palsy, multiple sclerosis and seizures are also postulated to share the common prenatal causative pathways [6]. Therefore, provoking the maternal immune response during pregnancy is utilized to develop animal models expected to mimic behavioral symptoms and pathophysiological mechanisms relevant for disorders linked with prenatal disruptions, such as schizophrenia and autism [7].

Lipopolysaccharide (LPS), a cell wall component of gram negative bacteria, administered intraperitoneally to pregnant rodents produces febrile response and cytokine induction, thus interfering with the balance between pro- and anti-inflammatory cytokines that is essential for the efficient neurodevelopment in fetus [8]. Data on LPS concentration and cytokine levels in maternal blood, placenta and fetal tissue suggest that effects of prenatal LPS administration are not mediated directly by LPS, but via indirect actions at the level of the maternal circulation or placenta [9]. The dosage and timing of LPS administration optimal for reaching cytokine induction devoid of maternal or fetal mortality were investigated thoroughly [10]. However, *in utero* brain development in rodents, due to a short gestation, offers a relatively narrow time window for precise management of immune insult. Thus, although protocols of maternal immune activation urge for outcome replicability and reproducibility, a spectrum of behavioral abnormalities is possible [7]. This is not at all unexpected, having in mind that LPS administrated on embryonic day 15 (E15) dysregulated as many as 3285 genes in rat fetal brain and induced widespread down-regulation of critical neurodevelopmental genes [11]. In a recent review of limitations in schizophrenia and autism models, it was proposed that acute prenatal neuroinflammation may predispose and contribute to pathological features shared by both disorders, whereas schizophrenia- and autism-specific phenotypes may be governed by subsequent latent and persistent inflammation, respectively [12].

An abundance of morphological and neurochemical abnormalities elicited by LPS administration to rats was found in the hippocampus, prefrontal cortex, nucleus accumbens, dorsal striatum and other regions related to schizophrenia and autism [13]. Data on behavioral disturbances induced by prenatal LPS treatment are extensive, but also hampered by ambiguity, which makes it difficult to establish the much needed face validity of the model [7]. This extensive diversity of behavioral reports will be carefully discussed in comparison with the results obtained in the present study, designed to probe symptoms related to traditional schizophrenia classification and in part overlapping with autism symptomatology, with employment of three behavioral paradigms: baseline and amphetamine induced locomotion (positive), social interaction (negative) and Morris water maze (cognitive symptoms). Tackling with the gender- and time-related differences in terms of onset of disturbances [12], we set this study to investigate behavioral abnormalities in Wistar offspring of both genders, exposed to the relevant dose of LPS in late embryogenesis [10], at their adolescent and young adult age.

2. Material and methods

2.1. Animals and LPS administration

All procedures in the study conformed to EEC Directive 86/609 and were approved by the Ethical Committee on Animal Experimentation of the Faculty of Pharmacy in Belgrade. Male and female Wistar rats were obtained from Military Farm (Belgrade, Serbia) at the age of two months. The rats were housed separately in male and female animal rooms for several days of acclimatization before a male and a female were joined in a single cage. Vaginal smears were taken daily at 9 am and if found positive for spermatozoa females were separated from the males. To avoid social isolation context pregnant dams were housed three per cage until gestation day 19 and then individually until delivery.

On gestation days 15 and 16 pregnant dams were treated intraperitoneally either with LPS (from *Escherichia coli*, serotype 0111:B4, Sigma L2630) at dose of 100 μg/kg per day, or with 0.9% saline (2 mL/kg per day). After delivery offspring were let with their mothers undisturbed until postnatal day 21 and then weaned and separated by gender. Thus, we obtained four testing groups of animals that were used in our experiments and in further text they will be referred to as: LPS males, LPS females, control males and control females.

The rats were housed in transparent plastic cages, up to five and not less than two per cage, and pups from different litters were not mixed. Animals had free access to food pellets and tap water. The temperature of the animal room was 22 ± 1 °C, the relative humidity 40–70%, the illumination 120 lx, and the 12/12 h light/dark period (light on at 6:00 h). All handling and testing took place during the light phase of the diurnal cycle.

2.2. Measurement of cytokine induction in maternal blood

Two hours after LPS or saline administration on gestational day 15, maternal tail blood was collected into heparinized tubes, centrifuged (5000 × *g*, 10 min at 4 °C) and plasma was collected and stored at −80 °C. TNF- a and IL-6 levels were determined using commercial ELISA kits (Rat TNF alpha ELISA Ready-SET-Go!®, eBioscence, USA and IL-6 ELISA Kit, Rat, Life Technologies, USA). The detection limits were 16 pg/ml for TNF-α and 23.5 pg/ml for IL-6.

2.3. Behavioral testing

Behavioral testing was performed at postnatal days 40 and 60 (P40 and P60). To reduce the litter effect experimental groups were formed to include pups from five to ten litters (at least two pups per litter). Care was taken in all experiments to counterbalance the test order across prenatal treatment and gender conditions. Offspring selected for social interaction experiment were kept in distant cages on different shelves in animal room.

2.3.1. Baseline and amphetamine induced locomotor activity

Locomotor activity testing was conducted in four blurred plexiglas chambers (40 × 25 × 35 cm) under dim red light (20 lx). Both prenatally challenged and control animals were tested simultaneously, but males and females were not tested at the same time. Testing was performed in three consecutive trials. In the first trial, a single rat was placed in the apparatus for 30 min of habituation. This was followed by saline application (*i.p.*) and rat's activity was recorded in the second 30-min trial to examine whether injection stressor *per se* induced locomotor response. Finally, the third trial started with an amphetamine administration (0.5 mg/kg, *i.p.*)

* Corresponding author. Fax: +381 11 3972840.
E-mail address: miroslav@pharmacy.bg.ac.rs (M.M. Savić).