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Neonatal immune activation by lipopolysaccharide causes inadequate emotional responses to novel situations but no changes in anxiety or cognitive behavior in Wistar rats

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ABSTRACT

Infection during the prenatal or neonatal stages of life is considered one of the major risk factors for the development of mental diseases such as schizophrenia or autism. However, the impacts of such an immune challenge on adult behavior are still not clear. In our study, we used a model of early postnatal immune activation by the application of bacterial endotoxin lipopolysaccharide (LPS) to rat pups at a dose of 2 mg/kg from postnatal day (PD) 5 to PD 9. In adulthood, the rats were tested in a battery of tasks probing various aspects of behavior: spontaneous activity (open field test), social behavior (social interactions and female bedding exploration), anxiety (elevated plus maze), cognition (active place avoidance in Carousel) and emotional response (ultrasonic vocalization recording). Moreover, we tested sensitivity to acute challenge with MK-801, a psychotomimetic drug. Our results show that the application of LPS led to increased self-grooming in the female bedding exploration test and inadequate emotional reactions in Carousel maze displayed by ultrasonic vocalizations. However, it did not have serious consequences on exploration, locomotion, social behavior or cognition. Furthermore, exposition to MK-801 did not trigger social or cognitive deficits in the LPS-treated rats. We conclude that the emotional domain is the most sensitive to the changes induced by neonatal immune activation in rats, including a disrupted response to novel and stressful situations in early adulthood (similar to that observed in human patients suffering from schizophrenia or autism), while other aspects of tested behavior remain unaffected.

1. Introduction

Mental diseases such as schizophrenia and autistic spectrum disorder (further referred to here simply as “autism”) in human population have serious consequences on patients’ quality of life including social and working abilities [1]. Both disorders share some risk factors and also exhibit some comparable symptoms, such as impairment in social interactions and communication, abnormal thinking, cognitive deficits, disrupted emotional states or abnormal behavioral responses to stressful situations, and difficulties in adapting to novel situations [2]. The exact etiology of these disorders is often unknown, which complicates the search for new effective therapies.

Both schizophrenia and autism are regarded as neurodevelopmental disorders [2–4]. Several lines of evidence have suggested that in addition to genetic mutations, pre-, peri- or postnatal environmental factors also contribute to the development of mental disorders [3,5,6]. For instance, dysregulation of the immune system could lead to a complex brain development changes [7] followed by abnormal behavior [8]. Immune system abnormalities have repeatedly been found in patients suffering from schizophrenia or autism [9–12]. Studies with human patients as well as animal models have shown that viral or bacterial infections occurring in critical periods of early life development are

Abbreviations: ACQ, acquisition; ANOVA, analysis of variance; CB1, cannabinoid receptor 1; GABA, γ-aminobutyric acid; GEE, generalized estimating equations; HPA, hypothalamus-pituitary-adrenal axis; i.p., intraperitoneal application; LPS, lipopolysaccharide; MaxT, maximum time of avoidance; NMDA, *N*-methyl-D-aspartate; PD, postnatal day; poly(I:C), polyinosinic:polycytidylic acid; REV, reversal; RM ANOVA, repeated-measure analysis of variance; USV, ultrasonic vocalization

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probably involved in the pathogenesis of schizophrenia as well as autism [7,13–18]. Maternal influenza virus, viral RNA analog polyinosinic:polycytidylic acid (poly I:C) and bacterial endotoxin lipopolysaccharide exposure during sensitive periods are all associated with neuropsychiatric symptoms resembling autism or schizophrenia in adult rodent offspring, with proinflamamtory cytokines implied to play a key role in the pathogensis [19].

Lipopolysaccharide (LPS) is an endotoxin released from the cell wall of gram-negative bacteria following cell division or lysis, and it causes the release of pro-inflammatory mediators from immune cells [20] and microglial activation [21]. Significant activation of the microglia in LPS models has been found in brain areas such as the hippocampus, cerebral cortex or thalamus [22–24].

Early immune stimulation by LPS alters the levels of neurotransmitters in adult animals [14,25–28] and induces the release of pro-inflammatory cytokines [28,29]. It also activates the hypothalamus-pituitary-adrenal (HPA) axis, and the resulting enhanced glucocorticoid levels might also contribute to disturbed brain development [30]. Such HPA axis disruption by infection stress could contribute to cognitive function impairments as a result of interactions with the dopaminergic system, and the role of glucocorticoids in cognitive deficits has also been demonstrated in some patients, e.g. those suffering from schizophrenia (see review [31]).

People with schizophrenia are also hypersensitive to amphetamine and psychotomimetic drugs [32,33]. In animal models, it has been shown that rats exposed to LPS in the prenatal period are more sensitive to the locomotor-stimulating effects of amphetamine [34], and the response to MK-801 is also exacerbated [14].

Our rat model used in this study was prepared by application of LPS in early stage of life, from the fifth to ninth postnatal days, which corresponds to the third trimester of human pregnancy [35]. Previously, our group demonstrated that the manipulation reduced overall hippocampal volume [26] and decreased number and branching of astrocytes in CA1 area of the hippocampus in adult animals [36] which could indicate an association with possible memory or cognition deficits. Kubesova et al. [26] also found in this model alterations in the neurotransmitter levels which could be connected to emotional changes, and additionaly astrogliosis and activated kynurenine catabolic pathway of tryptophan metabolism which all that have been associated with mental disorders [26]. On the behavioral level, Tejkalová et al. [36] found prepulse inhibition deficit in early adulthood of the rats providing a connection to schizophrenia. Prepulse inhibition deficit in LPS-mediated models was described repeatedly [14,24,30,36], however, the effect on other aspects of behavior is still not fully clear. The inconsistency of results in previous studies could be probably explained by the diverse protocols of LPS application as well as other factors. As a consequence, there are ambiguous findings concerning the role of prenatal/neonatal infections in the etiology of mental diseases.

The aim of the present work was to study the behavioral effects of the early postnatal exposure of rats to LPS using tests taxing spontaneous activity in novel environment, anxiety, social behavior, cognition and emotional state, with the hypothesis that this model of early-life infection would lead to impairments in these behavioral domains. Furthermore, we hypothesized that LPS-treated rats would exhibit increased sensitivity to the systemic application of MK-801 in adulthood, paralleling the situation in human patients and also in the animal model with prenatal LPS exposure.

2. Materials and methods

2.1. Animals

Forty-seven adult male Wistar/Hann rats (Velaz Ltd., Czech Republic) were used in the experiments. The animals were kept in groups of 2–3 individuals in standard Plexiglas boxes (44 × 28 × 23 cm), in an air-conditioned room (22 °C) with a standard 12/12 light/dark cycle, with *ad libitum* access to water and food. After an acclimatization period at the Institute of Physiology, CAS, animals were handled for five days (handling included habituation to human touch, holding and manipulations by the experimenters for 5–10 min per day). All experiments were approved by the local Committee for animal protection (Project of Experiments No. 136/2013) and local Committee MHCR (No. 17/2012) complied with the Animal Protection Act of the Czech Republic, EU directive (2010/63/EC).

2.2. Model preparation

Six female rats with litters were housed individually, in standard Plexiglas boxes. Only male offspring (n = 9 per litter) were used for the experiments. The pups were treated daily from postnatal day (PD) 5 to PD 9 with either lipopolysaccharide (LPS, *Escherichia coli*, serotype 026:B6; Sigma–Aldrich) at a dose of 2 mg/kg (LPS group) or with an equivalent amount of 0.9% saline (control group) administered intraperitoneally (i.p.). Littermates were randomly assigned to LPS or control groups in a balanced manner (i.e. half of the males from each litter received LPS and the other half received saline). The pups were weaned on PD 28. The behavioral screening was done on 2-month old animals or older (see order of the tests and numbers of animals in Table 1).

2.3. Open field test

We tested 23 animals in total (11 LPS, 12 controls) at the age of 2 months in an open field test [37], using a chipboard white-colored square box (70 × 70 cm). One wall of the box was transparent, and the animals were recorded by a camera at an oblique angle. The animals were tested for 5 min during the late afternoon (15:00 – 19:00). We analysed **rearing** as a measure of spontaneous exploratory activity, and **grooming** as a measure of comfort or conflict behavior.

2.4. Social interactions

A social interaction task was used to measure social behavior, because social impairment is among the core symptoms of autism and is also present in schizophrenia. Two rat males from the same group (LPS or control) were placed into the white-colored square box previously used for the open field test and were recorded by the oblique-view camera for 10 min. We analyzed the frequency and total duration of several categories of social behavior: **anogenital exploration**, **following the social partner**, **climbing over the partner** (UP), **crawling under the partner** (DOWN) and **non-anogenital sniffing with other social contact**. **Rearing** and **self-grooming** were considered as non-social behavior. Twenty animals were used in total and each animal

Table 1
Order of the tests and numbers of tested animals. LPS = lipopolysaccharide, MK = MK-801, Sal = saline.

Test order	Test	Number of animals per group			
		Sal		LPS	
1	Open field test	n = 12		n = 11	
2	Social interactions	n = 10		n = 10	
3	Female bedding exploration	n = 11		n = 10	
4	Carousel maze - acquisition	n = 24		n = 23	
6	Elevated plus maze	n = 12		n = 11	
		Sal + Sal	Sal + MK	LPS + Sal	LPS + MK
5	Carousel maze - reversal	n = 10	n = 10	n = 9	n = 10
7	Social interactions after drug treatment	n = 8	n = 4	n = 4	n = 4