Behavioural Brain Research 304 (2016) 1–10

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Neonatal proinflammatory challenge in male Wistar rats: Effects on behavior, synaptic plasticity, and adrenocortical stress response

Anna Tishkina[1], Mikhail Stepanichev[1], Irina Kudryashova, Sofia Freiman, Mikhail Onufriev, Natalia Lazareva, Natalia Gulyaeva*

Department of Functional Biochemistry of the Nervous System, Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow 117485, Russia

HIGHLIGHTS

- Neonatal proinflammatory stress (NPS) induces increased anxiety in adolescent and adult rats.
- NPS induces development of depression-like behaviors in adult rats.
- NPS modulates corticosterone response to stress in adolescent and adult rats.
- NPS impairs hippocampal synaptic plasticity in adolescent rats.
- NPS enhances fear conditioning in adult rats.

ARTICLE INFO

Article history:
Received 23 August 2015
Received in revised form 31 January 2016
Accepted 1 February 2016
Available online 3 February 2016

Keywords:
Neonatal proinflammatory stress
Lipopolysaccharide
Anxiety
Depression
Contextual fear conditioning
Long term potentiation
Hippocampus
Hypothalamic–pituitary–gonadal axis
Corticosterone
Maturation

ABSTRACT

Effects of neonatal proinflammatory stress (NPS) on the development of anxiety and depressive-like behavior, stress responsiveness, hippocampal plasticity and conditioned fear response were studied in adolescent and adult male Wistar rats. On PND 3 and PND 5, the pups were subcutaneously injected with bacterial lipopolysaccharide (LPS, 50 μg/kg). In the open field test, signs of increased anxiety were demonstrated in adolescent (PND 32), but not in adult (PND 101) rats. In the elevated plus maze, no changes could be detected in adolescent rats, however, in the adults the number of entries into the open arms decreased suggesting increased anxiety after NPS. Signs of "behavioral despair" in the forced swim test, expressed in adolescent rats as a trend, became significant in the adults indicating depression-like behavior. In the majority of brain slices from PND 19-PND 33 rats subjected to NPS, deficit of LTP in the hippocampal CA1 field was detected, this deficit being associated with the impaired mechanisms of LTP induction. In the adult rats, NPS enhanced fear conditioning promoting improved formation of the novel context-foot shock association in the contextual fear conditioning paradigm without effect on cued fear conditioning. NPS significantly impaired functioning of the hypothalamic–pituitary–adrenal axis (HPAA), resulting in an elevated corticosterone level maintained in the adolescents but not in the adults and in modified corticosterone response to behavioral sub-chronic stress in both adolescent and adult rats. Thus, NPS induces "perinatal malprogramming" resulting in development of depression-like behaviors, associated with abnormalities in functioning of the HPAA, impaired hippocampal neuroplasticity (LTP) and changes in hippocampus-dependent memory formation.

1. Introduction

Treatment of neonate rodent pups with bacterial lipopolysaccharide (LPS), specifically on postnatal days (PNDs) 3 and 5, a neonatal proinflammatory stress (NPS), is regarded as a model of impaired "perinatal programming", a process resulting in considerable physiological and behavioral alterations [1–3]. This

Abbrevations: CRH, corticotrophin releasing hormone; CORT, corticosterone; EPM, elevated plus maze; Fepsp, field excitatory postsynaptic potential; FST, forced swim test; HPAA, hypothalamic–pituitary–adrenal axis; GABA, γ-aminobutyric acid; IL, interleukin; LPS, lipopolysaccharide; LTP, long term potentiation; NPS, neonatal proinflammatory stress; PND, postnatal day; OFT, open field test.

* Corresponding author at: Department of Functional Biochemistry of the Nervous System, Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Butlerova str., 5A, Moscow 117485, Russia. Tel.: +7 495 9524007; fax: +7 495 9524007.
E-mail addresses: nata_gul@yahoo.com, nata_gul@pisem.net (N. Gulyaeva).

[1] These authors equally contributed to the study.

http://dx.doi.org/10.1016/j.bbr.2016.02.001
0166-4328/

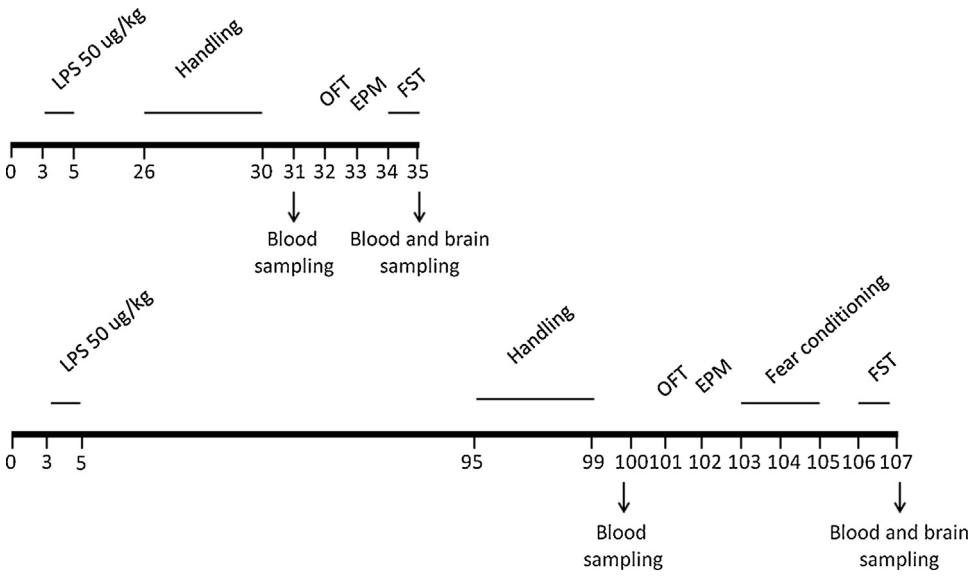

Fig. 1. Experimental design. Upper line—manipulations with adolescent rats; lower line—manipulations with adult rats.

phenomenon encompasses the role of the intrauterine and early postnatal environment in the predisposition to and onset of adult disease (perinatal malprogramming) [4,5]. Most reproducible behavioral outcomes in the adulthood include increased anxiety-like behaviors, demonstrated as more time spent in the closed arms and fewer entries to the open arms of an elevated plus maze (EPM), reduced exploratory behavior in the holeboard apparatus, and increased risk assessment behavior in the open field test (OFT) [1,6–8]. The physiological abnormalities include the impaired body weight gain [9,10] as well as disturbed autonomic and endocrine regulation [9]. These effects are often differently expressed in males and females [9,11]. In addition to increased anxiety, the animals treated with LPS in the neonatal period may exhibit depression-like behavioral features as adults; however, only sporadic studies reported this effect [9].

Neuropsychological deficits associated with anxiety disorders and depression are often considered just as epiphenomena of age, poor motivation, inattention or response bias. However, several studies demonstrate persistent cognitive impairment upon recovery in mood disorder, indicating association of high anxiety and depression with impairments of cognitive functions [12,13]. Depression is associated with a number of deficits in episodic memory and learning whereas implicit memory tasks appear to be spared (see Ref. [12] for review). Neonatal immune challenge has significant impact not only on anxiety and depressive-like behavior, but also on cognitive functions in adult animals. Administration of bacterial *Escherichia coli* suspension on PND 4 resulted in the improved rate of hippocampal-dependent task acquisition in a Morris water maze, while impairing flexibility of the same task and dentate gyrus network activation [14]. In this model, impairments in learning and memory become more evident in adult animals after additional immune challenge with LPS [15,16]. However, Kohman et al. [17] reported that neonatal LPS administration on PNDs 4 and 5 disrupted two-way active avoidance learning in male, but not in female mice in the absence of additional LPS administration in the adulthood. LPS treatment on PNDs 5 or 30 impaired memory on the location of a hidden platform in Morris water maze and in contextual fear conditioning paradigm [18]. This difference in the capability of LPS alone to induce much more expressed effects as compared to early bacterial infection, specifically in hippocampal-dependent behavior, are probably related to a larger and broader inflammatory response, which LPS evokes in the hippocampus [19].

Although the mechanisms of enhanced anxiety and depressive-like behavior due to proinflammatory stimulation early in life remain to be elucidated, the effects of such treatment on the hippocampus may be involved. Another important reason is an abnormal development of the hypothalamic–pituitary–adrenal axis (HPAA) and hypothalamic–pituitary–gonadal axis induced by exposure to this proinflammatory treatment [10]. Proinflammatory stimulation is a strong stressor for a developing organism. Neonatal LPS treatment produced a persistent increase in tyrosine hydroxylase phosphorylation and activity in the adrenal gland of adult rats [10]. This was accompanied by alterations in circulating corticosterone (CORT) reported in adult animals treated with LPS as neonates, this disturbance becoming particularly evident during the exposure of these animals to an additional acute stressor in adulthood [20–22]. The alterations in the circulating CORT started as early as several hours after the immune challenge and continued in the adulthood. Increased corticotropin-releasing hormone (CRH) mRNA levels in the hypothalamus in male rats and decreased glucocorticoid receptor density in the hypothalamus, hippocampus and frontal cortex of both male and female rats have been reported [23].

In general, the process of "perinatal programming" by the early life exposure to an inflammatory insult has been suggested to alter later response of the HPAA. Molecular mechanisms of this phenomenon are not completely understood, however, it is clear that proinflammatory cytokines, such as interleukin (IL)-1β, tumor necrosis factor-α (TNFα) and IL-6 are primary mediators of HPAA-mediated response to peripheral inflammatory challenges [24]. Additionally, hippocampal damage may weaken the inhibitory influences on the hypothalamus, mediated by descending γ-aminobutyric acid (GABA)-ergic projections of neurons of the bed nucleus stria terminalis [25,26]. The data reported by several groups suggest an involvement of GABA insufficiency in LPS-induced activation of the HPAA [10,27]. In adult male rats, administration of GABA [28] or a GABA-A receptor agonist [29] prior to LPS injection has been shown to reduce plasma CORT levels and CORT accumulation in the hippocampus as compared to animals treated with LPS alone. Neonatal LPS exposure on PNDs 7 and 9 resulted in a selective decrease of GABA containing interneurons in hippocampal regions of adult rats [30] indicating altered GABA signaling induced by neonatal immune activation. Noteworthy, neonatal LPS treatment induced a significant modification in the composition of dopamine, serotonin, and glutamate recep-