DOI 10.1007/s11055-020-01025-7
Neuroscience and Behavioral Physiology, Vol. 50, No. 9, November, 2020

Changes in Behavior and the Expression of Ionotropic Glutamate Receptor Genes in the Brains of Adult Rats after Neonatal Administration of Bacterial Lipopolysaccharide

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Translated from Rossiiskii Fiziologicheskii Zhurnal imeni I. M. Sechenova, Vol. 106, No. 3, pp. 356–372, March, 2020. Original article submitted December 23, 2019. Revised version received January 21, 2020. Accepted January 21, 2020.

Many studies have shown that early experience, particularly of neonatal infections, has a role in forming high anxiety levels in later life. One of the mechanisms of these changes may consist of impairments to the functional activity of ionotropic glutamate receptors associated with rearrangements in their subunit composition. The aim of the present work was to study measures of anxiety and levels of expression of genes for NMDA receptor (*Grin1*, *Grin2a*, *Grin2b*) and AMPA receptor (*Gria1*, *Gria2*) subunits in the medial prefrontal cortex, ventral and dorsal parts of the hippocampus in adult rats which at an early age had received bacterial lipopolysaccharide (LPS) at doses inducing the development of neuroinflammatory processes. Gene expression was studied by real-time reverse transcription PCR (qRT-PCR). Administration of LPS at doses of 25 and 50 μg/kg to male Wistar rats on days 15, 18, and 21 of life induced increases in the expression of genes for proinflammatory cytokines IL-1β and tumor necrosis factor in parts of the hippocampus. Increases in the expression of the *Grin2b*, *Gria1*, and *Gria2* genes in the ventral parts of the hippocampus were seen three months after LPS administration (after injection of 50 μg/kg LPS), as were increases in expression of the *Gria2* gene in the dorsal part of the hippocampus (after injection of 25 μg/kg LPS). These changes were accompanied by impairment to exploratory behavior in the open field test and decreased anxiety levels in the elevated plus maze. These studies showed that administration of bacterial LPS in early postnatal ontogeny leads to time-delayed changes in the expression of genes for NMDA and AMPA receptor subunits in the hippocampus and the associated forms of behavior.

Keywords: early development, brain, inflammation, lipopolysaccharide, anxiety, NMDA receptor, AMPA receptor.

Anxiety disorders are the commonest mental disorders throughout the world: data from different studies indicate that from 3.8% to 33.7% of adult people encounter such problems at least once during their lives [1, 2]; the molecular mechanisms of predisposition to developing anxiety disorders remain poorly studied.

Many studies have indicated a role for early experience, particularly of prenatal and neonatal stresses and infectious disease, in forming high anxiety levels in later life [3, 4].

An experimental model of neonatal infectious diseases is provided by administration of bacterial lipopolysaccharide (LPS, endotoxin) to animals; LPS is an element of the cell wall of Gram-negative bacteria. Binding to Toll-like receptors type IV (TLR4), LPS induces the synthesis of proinflammatory cytokines – interleukins (IL)-1β and -6 and tumor necrosis factor (TNF) – in cells of the immune and nervous systems [5], which leads to the development of a prodromal syndrome including increased body temperature, impairments to digestive and exploratory motivations, suppression of social activity, a predominance of slow-wave sleep, and activation of the hypothalamo-hypophyseal-adrenal system [6]. These effects were short-lived in adults after administration of moderate endotoxin doses, though administration of

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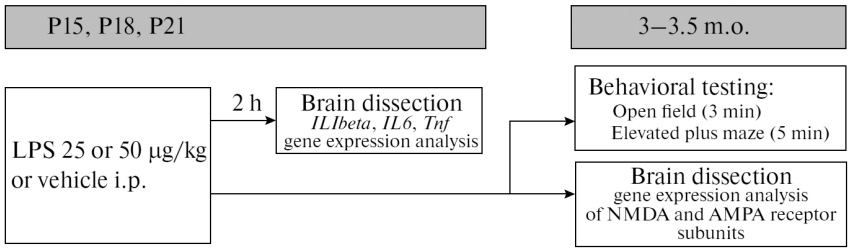

Fig. 1. Experimental scheme. Bacterial LPS was given to rats on days 15, 18, and 21. Expression of genes for proinflammatory cytokines was studied in the medial prefrontal cortex (mPFC) and dorsal and ventral hippocampus (DH, VH) 2 h after the last injection. Levels of exploratory and anxiety behavior were assessed in the open field and elevated plus maze tests at age 3–3.5 months. Expression of the genes for NMDA and AMPA receptor subunits were determined in the mPFC, DH, and VH in a separate group of animals at the same age.

the same endotoxin doses in early ontogeny could produce long-lasting adverse effects on CNS functions. In particular, the offspring of rats given LPS during pregnancy and rat pups in the first days of life were found in adolescence to display elevated locomotor activity [7], to develop depression-like and anxious behavior [8–10], and in adulthood to display depression-like changes in behavior [10–12].

It has been suggested that one of the factors in the occurrence of psychoemotional disorders associated with negative environmental influences at early age may be impaired formation of NMDA and AMPA glutamate receptors in CNS cells [13]. The role of these receptors in controlling neuroplasticity, learning and memory processes is widely recognized [14–17]. However, the involvement of NMDA and AMPA receptors in the pathogenesis of bipolar disorders is also discussed [18–20], as the level of expression of NMDA receptors in the hippocampus correlates with measures of anxiety [21], while intrahippocampal administration of their agonists modulates anxiety levels in experimental animals [22].

The functional activity of NMDA and AMPA receptors is directly related to their subunit composition [23–25]. The NMDA receptor complex is a heterotetramer consisting of an obligatory GluN1 subunit and variant GluN2(a–d) or GluN3(a,b) subunits, such that there is great functional and regional variation in NMDA receptors [24]. NMDA receptor subunits are encoded by the *Grin1*, *Grin2a-Grin2d*, *Grin3a*, and *Grin3b* genes. AMPA receptors consist of four subunits, GluA(1–4) and are usually dimers of two GluA2 dimers and two other (GluA1, GluA3, or GluA4) subunits [26]. AMPA receptor subunits are encoded by the *Gria1-Gria4* genes. GluA2-containing receptors, in contrast to those not containing these subunits, are impermeable to calcium ions [27].

Early postnatal ontogeny is a critical period in the formation of the subunit composition of NMDA and AMPA receptor complexes in the rat brain. Maturation of receptors is apparent as changes in their construction, the process having different dynamics in different parts of the brain and different neuron subtypes [28–32]. Significant age-related rearrangements in the subunit composition of NMDA receptors take place in the rat hippocampus at 2–3 weeks of life [33–35]. These are expressed particularly as increases in GluN2a production and, as a result, increases in the proportion of NMDA receptors containing this subunit. AMPA receptors in cortical neurons mature at essentially the same time [36, 37]. Immature synapses contain calcium-permeable AMPA receptors, which are replaced by GluA2-containing calcium-impermeable receptors as the brain matures [38]. Data on the influences of neonatal infections on the formation of the subunit composition of NMDA and AMPA receptors during their critical maturation period are few and scattered.

The present study assessed measures of anxiety and levels of expression of the genes for NMDA and AMPA receptor subunits in brain cells in adult rats given bacterial LPS during the third week of life – the period of active formation of the subunit composition of NMDA and AMPA receptors.

Methods. ***Study system and experimental design.*** Experiments were performed on Wistar rat pups in compliance with the humanitarian principles laid out in European Community Directive No. 86/609/EC and approved by the local Ethics Committee of the Institute of Evolutionary Physiology and Biochemistry. Animals were kept in the institute animal house as stress associated with transporting pregnant females could have significant influences on the behavior of the offspring. Rat pups were kept with their mothers to age 30 days, after which they were separated and kept in standard conditions. Studies used a total of 73 rats from 18 litters (31 in behavioral and 42 in biochemical experiments). The numbers of rat pups in litters varied (8 or 9 animals). Female pups were by necessity left in the litter but were not used in experiments. Male pups from each litter were divided into three groups: animals of two groups received i.p. LPS at the moderately pyrogenic doses of 25 or 50 μg/kg on days 15, 18, and 21 of life, while rats of the control group received i.p. administration of the same volume of apyrogenic physiological saline at the same times. These LPS doses were moderately pyrogenic, as they increased body temperature by 0.6–1.9°C in most animals 2–4 days after administration, as determined in preliminary experiments. LPS was given three times to model frequent childhood infections. During administration, rat pups were parted from their mothers for no more than 15 sec. The experimental scheme is shown in Fig. 1.

The experimental model used here was characterized, particularly for evidence of the ability of the LPS doses used to trigger inflammatory processes in the brain, by decapitating a proportion of the experimental and control animals 2 h after the last injection for subsequent determination of the expression of genes for proinflammatory cytokines IL-1β, IL-6, and TNF in brain cells.

The long-term effects of LPS administration in the third week of life on the expression of the genes for NMDA and AMPA receptor subunits and glutamate-dependent forms of behavior were evaluated in individual groups of experimental and control rats at age 3–3.5 months.

Behavioral testing. *Open field*. Orientational-exploratory behavior, locomotor activity, and anxiety levels in rats were evaluated in the open field test, which was a round arena of diameter 100 cm with sides of height 40 cm and a double floor, with 16 openings of diameter 3.5 cm and depth 1 cm, which were termed "holes." Testing was performed once, for 3 min. Experiments were run from 18:00 to 22:00 with an illumination level of 25 lx. After each rat, the arena was wiped with 0.6% hydrogen peroxide to remove odors and then with water to remove peroxide, after which the arena was wiped dry. The animals' behavior was assessed in terms of the durations of behavioral patterns of exploratory behavior (rearings with support, exploration of holes), anxiety (vertical rearings without support, grooming), and locomotor activity (locomotion, movement on the spot).

Elevated plus maze. Anxiety and exploratory behavior were also assessed in the elevated plus maze, which consisted of two open and two closed arms, 50 cm long and 10 cm wide, positioned perpendicular to each other. The heights of the walls of the closed arms was 40 cm and the height of the apparatus from the floor was 60 cm. Animals were tested once, for 5 min, at illumination levels of 25 lx for the open arms, 5 min after testing in the open field. Anxiety in the rats were assessed in terms of the total time spent in the open arms and exploratory activity was assessed in terms of the total duration of peeking from the closed arms.

Determination of the durations of behavioral acts in both tests was in real time using the ethology program Field 4, developed at the Department of Physiology, Pavlov Institute of Experimental Medicine.

Assessment of gene expression levels. Gene expression levels were evaluations in terms of the contents of mRNA for the genes of interest relative to the content of mRNA for the reference gene, which was the housekeeping gene *Gapdh*. Animals aged 3–3.5 months were decapitated and brains were removed and immediately frozen in liquid nitrogen and stored at –70°C until biochemical studies were run.

Total RNA was extracted from the brain structures of interest and used in reverse transcription reactions followed by polymerase chain reactions (RT-PCR) in real-time conditions.

RNA extraction. The medial prefrontal area of the cortex (mPFC) and the dorsal and ventral parts of the hippocampus (DH, VH) were harvested from frozen brains on slices using a Thermo Scientific Microm HM525 cryostat microtome at –20°C using the scheme described in the *Rat Brain Atlas in Stereotaxic Coordinates* [39], with boundaries rostral and caudal relative to the bregma from 4.20 to 2.52 mm for the mPFC, from –2.64 to –5.28 mm for the DH, and from –4.56 to –5.28 mm for the VH.

Total RNA was extracted from brain structures by single-step acid guanidine-isothiocyanate-phenol-chloroform extraction following the protocol provided with the TRI reagent. Immediately after extraction, brain structures were placed in TRIzol (TRI Reagent®; Molecular Research Centre Inc., USA) and homogenized. The resulting solution was then supplemented with chloroform. Ten minutes after intense mixing for 1 min, the solution was centrifuged at 12000 *g* for 30 min and the resulting upper aqueous phase, containing RNA, was collected into a separate tube and left for 12–15 h with the same volume of isopropanol at +4°C. This solution was then centrifuged at 12000 *g* for 15 min and the resulting RNA pellet was placed in 70% ethanol for storage at –20°C until reverse transcriptase-polymerase reactions were run to produce complementary DNA (cDNA) from the RNA template.

RT-PCR. Dried RNA pellets were dissolved in diethylpyrocarbonate-treated deionized water. RNA concentrations were measured with a Nano Drop 2000 spectrophotometer (Thermo Fisher Scientific Inc., USA) to determine optical density at a wavelength of 260 nm. Reverse transcription was run using 1 μg of RNA from each sample. Oligo-dT primers were annealed to RNA at +70°C for 5 min. After brief cooling and centrifugation, tubes were supplemented with reaction mix containing reverse transcription buffer, nucleotides, RNase inhibitors, and M-MLV reverse transcriptase using the quantities specified in the M-MLV reverse transcriptase manufacturer's protocol (Promega, Fitchburg, USA). Tubes were placed for 70 min in a C1000 Touch™ thermal cycler (Bio-Rad Laboratories, USA) for cDNA synthesis reactions on the RNA template at +42°C. After stopping the reaction at +65°C for 10 min, the resulting cDNA samples were stored at –20°C until the real-time polymerase chain reaction was run.

PCR using TaqMan technology was by supplementation of 1 μl of cDNA samples with reaction mix containing Taq buffer, $MgCl_2$ solution, nucleotides, forward and reverse primers, probe, and Taq polymerase using the quantities specified in the TaqM polymerase manufacturer's protocol (Alkor-Bio, St. Petersburg, Russia). Amplification was run in a C1000 Touch™ thermal cycler with a CX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, USA) using the following program: a hot start at +95°C, 300 sec; denaturation at +95°C for 5 sec; primer annealing and elongation with recording of fluorescence at +60°C for 10 sec (50 cycles). Analysis of each sample was run in duplicate. A negative control without template was run, along with a negative reverse transcription control (RNA samples without reverse transcription in the RT reac-

TABLE 1. Nucleotide Sequence of Primers and Probes

Gene, (GeneBank), encoded protein	Nucleotide sequences (5′ → 3′): forward primer, reverse primer, probe	Reference
Gapdh (NM_017008)	TGCACCACCAACTGCTTAG GGATGCAGGGATGATGTTC HEX-ATCACGCCACAGCTTTCCAGAGGG-BHQ-1	[40]
IL1beta (NM_031512) Il-1β	CACCTCTCAAGCAGAGCACAG GGGTTCCATGGTGAAGTCAAC FAM-TGTCCCGACCATTGCTGTTTCCTAGG-BHQ-1	[41]
IL6 (NM_012589) Il-6	CAAGACCATCCAACTCATCTTG CACAGTGAGGAATGTCCACAAAAC FAM-TCGGCAAACCTAGTGTGCTATGCCTAAGCA-BHQ-1	
Tnf (NM_012675) TNF	CCAGGTTCTCTTCAAGGGACAA CTCCTGGTATGAAATGGCAAATC FAM-CCCGACTATGTGCTCCTCACCCACA-BHQ-1	
Grin1 (NM_012573) GluN1	GTTCTTCCGCTCAGGCTTTG AGGGAAACGTTCTGCTTCCA FAM-CGGCATGCGCAAGGACAGCC-BHQ-1	[42]
Grin2a (NM_012573) GluN2a	GCTACACACCCTGCACCAATT CACCTGGTAACCTTCCTCAGTGA FAM-TGGTCAATGTGACTTGGGATGGCAA-BHQ-1	[43]
Grin2b (NM_012574) GluN2b	CCCAACATGCTCTCTCCCTTAA CAGCTAGTCGGCTCTCTTGGTT FAM-AGACGCCAAACCTCTAGGCGGACAG-BHQ-1	
Gria1 (NM_031608) GluA1	TCAGAACGCCTCAACGCC TGTAGTGGTACCCGATGCCA ROX-TCCTGGGCCAGATCGTGAAGCTAGAAAA-BHQ-2	[44]
Gria2 (NM_017261) GluA2	CAGTGCATTTCGGGTAGGGA TGCGAAACTGTTGGCTACCT FAM-TCGGAGTTCAGACTGACACCCCA-BHQ-1	

tion mix). The nucleotide sequences of the primers and probes are shown in Table 1.

Amplification curves were analyzed in the Bio-Rad CFX Manager™ 2.1 program (Bio-Rad Laboratories, USA) using threshold curves. Primer effectiveness in multiplex PCR was checked in separate experiments. Relative quantities of mRNA in test samples assessed using the $2^{-\Delta\Delta Ct}$ method relative to the level of mRNA for the housekeeping gene *Gapdh* [45] and averaged values for the control group.

Statistical analysis. Statistical data processing was run in Microsoft Excel (Microsoft Corp., USA), SPSS Statistics 20 (IBM Corp., USA), and GraphPad Prism 8 (GraphPad Software Inc., USA). Normal distributions of datasets were evaluated using the Kolmogorov–Smirnov test. As many of the study parameters did not have normal distributions, groups were compared using the Kruskal–Wallis H test with the a posteriori Dunn test. Differences were regarded as significant at $p < 0.05$. Plots show medians and interquartile intervals.

Results. ***Administration of LPS at early age increases the level of expression of proinflammatory genes in the hippocampus.*** These experiments demonstrated increases in the level of expression of the interleukin-1β gene *IL1beta* in the dorsal and ventral areas of the hippocampus in rat pups 2 h after the final injection of LPS on day 21 of postnatal development as compared with control animals given physiological saline; a statistically significant increase in this parameter was also seen in the dorsal part of the hippocampus on administration of the lower dose of LPS, 25 μg/kg (physiological saline/LPS25 $p = 0.03$; physiological saline/LPS50 $p = 0.09$; pairwise comparison using Dunn's test, Fig. 2, *B*), and in the ventral hippocampus with both the higher LPS dose of 50 μg/kg (physiological saline/LPS25 $p = 0.13$; physiological saline/LPS50 $p = 0.01$; pairwise comparison using Dunn's test, Fig. 2, *C*). There was no change in this parameter in the medial prefrontal cortex after either LPS dose as compared with animals of the control group ($p > 0.05$, Kruskal–Wallis test, Fig. 2, *A*).

The level of expression of the interleukin-6 gene *IL-6* showed no significant change 2 h after administration of LPS into the mPFC or areas of the hippocampus ($p > 0.05$, Kruskal–Wallis test, Fig. 2, *D*–F*)*.

An increased level of expression of the tumor necrosis factor gene *Tnf* as compared with the control group was seen in the dorsal hippocampus of animals 2 h after injection of 50, but not 25, μg/kg of LPS (physiological saline/LPS25 $p = 0.51$; physiological saline/LPS50 $p = 0.03$; pairwise comparison using Dunn's test, Fig. 2, *H*), the quantity of mRNA for this gene in the VH being no different from that in controls after administration of both LPS doses

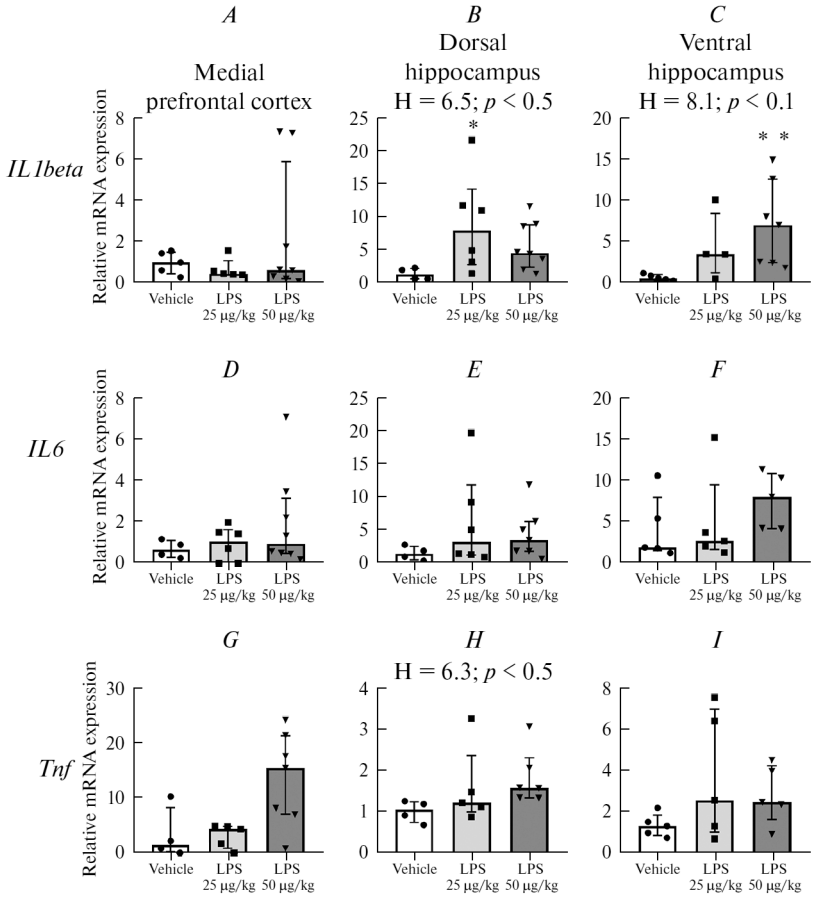

Fig. 2. Increases in levels of expression of genes for proinflammatory cytokines in the hippocampus after LPS administration in early age. H – Significant between-group differences, Kruskal–Wallis test; *$p < 0.05$; **$p < 0.01$ compared with control group, a posteriori Dun's test. $n = 4$–8. Data are presented as medians and interquartile intervals.

($p > 0.05$, Kruskal–Wallis test, Fig. 2, *I*). The increase in production of *Tnf* mRNA in the mPFC after administration of LPS did not reach statistical significance (H = 5.7, $p = 0.051$, Kruskal–Wallis test, Fig. 2, *G*).

Administration of LPS at early age leads to long-term impairments to orientational-exploratory behavior. Testing of animals in the open field revealed a reduction in exploratory activity in adult animals given LPS in week 3 as compared with animals of the control group, which was apparent as a decrease in the total duration of rearing with support (H = 6.10, $p = 0.047$, pairwise comparison, Dunn's test, physiological saline/LPS25, $p = 0.04$, Fig. 3, *A*). The same tendency was seen in relation to the total number or rearings with support, though the difference did not reach statistical significance (H = 5.75, $p = 0.057$). Further measures of exploratory behavior – the total duration of hole exploration in the open field and the duration of peeking from the open arms of the elevated plus maze – did not identify any between-group differences (H = 2.9, $p = 0.23$ and H = 2.9, $p = 0.24$, respectively, Kruskal–Wallis test, Fig. 3, *C*, *D*).

Anxiety in adult animals given LPS in the early period of postnatal ontogeny was lower than in control rats, which was apparent as an increase in the time spent in the open arms of the elevated plus maze (H = 12.87, $p = 0.002$, physiological saline/LPS50 $p = 0.004$, pairwise comparison, Dunn's test, Fig. 3, *F*). An increase in grooming time in animals given LPS did not reach the level of statistical significance (H = 4.3, $p = 0.12$, Kruskal–Wallis test, Fig. 3, *E*).

The duration of locomotor activity was decreased in rats given LPS 25 μg/kg (H = 7.20, $p = 0.028$, physiological saline/LPS25, $p = 0.051$, pairwise comparison, Dunn's test, Fig. 3, *G*). In terms of movement on the spot, there were no significant differences between animals of the three groups (H = 5.4, $p = 0.07$, Kruskal–Wallis test, Fig. 3, *H*). As clear qualitative impairments of motor functions in experimental rats were not seen, it can be suggested that the decrease in locomotion in the novel space was linked with decreased exploratory activity.

Thus, administration of LPS in the third week of life led to moderate impairment to exploratory behavior and reductions in the level of anxiety in the experimental animals.

Administration of LPS at early age induces long-term changes in the level of expression of the genes for NMDA and AMPA receptors in the hippocampus. PCR analysis of the quantities of mRNA for glutamate NMDA receptor subunit genes *Grin1*, *Grin2a*, and *Grin2b* and the *Grin2a*/

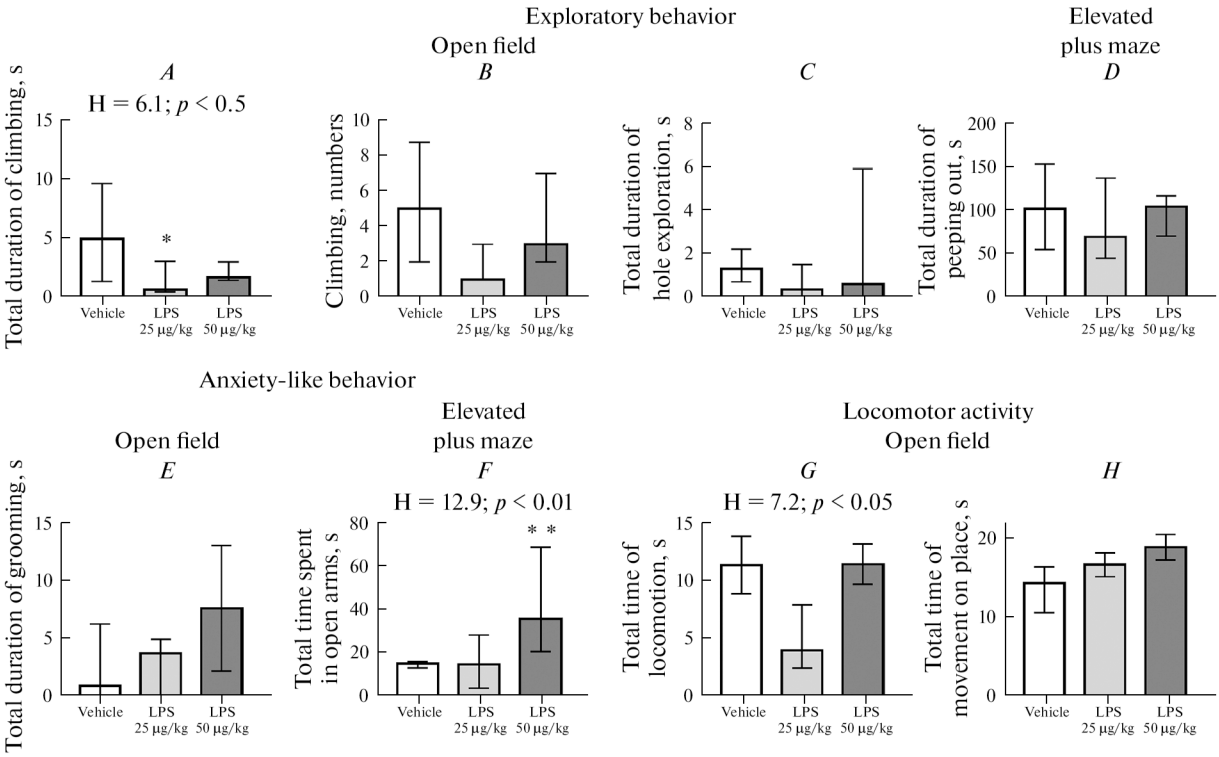

Fig. 3. Long-term changes in orientational-exploratory behavior in animals due to administration of LPS in early age. H – Significant between-group differences, Kruskal–Wallis test; *$p < 0.05$; **$p < 0.01$ compared with control group, a posteriori Dunn's test. $n = 6–14$. Data are presented as medians and interquartile intervals.

Grin2b ratio did not identify any differences in the mPFC or DH between the control group and groups of animals given each dose of LPS at early age (Kruskal–Wallis test, mPFC: *Grin1* H = 2.6, $p = 0.28$, Fig. 4, *A*; *Grin2a* H = 0.8, $p = 0.68$, Fig. 4, *D*; *Grin2b* H = 0.7, $p = 0.73$, Fig. 4, *G*; *Grin2a/Grin2b* H = 2.2, $p = 0.34$, Fig. 4, *J*; DH: *Grin1* H < 0.1, $p = 0.96$, Fig. 4, *B*; *Grin2a* H = 2.3, $p = 0.34$, Fig. 4, *E*; *Grin2b* H = 2.3, $p = 0.34$, Fig. 4, *H*; *Grin2a/Grin2b* H = 3.2, $p = 0.21$, Fig. 4, *K*).

The ventral area of the hippocampus also showed no differences between groups in terms of the level of expression of genes for the GluN1 and GluN2a subunits (Kruskal–Wallis test, *Grin1* H = 3.4, $p = 0.18$, Fig. 4, *C*; *Grin2a* H = 1.2, $p = 0.57$, Fig. 4, *F*). An increase in the level of production of mRNA for the GluN2b subunits in the VH in adult animals given 50 μg/kg LPS in week 3 as compared with the control group (physiological saline/LPS25 $p = 0.64$, physiological saline/LPS50 $p < 0.01$, pairwise comparison, Dunn's test, Fig. 4, *I*) wa seen, along with a decrease in the ratio of the mRNA quantities *Grin2a/Grin2b* in the ventral hippocampus of these animals as compared with controls (physiological saline/LPS25 $p = 0.12$; physiological saline/LPS50 $p < 0.01$, pairwise comparison, Dunn's test, Fig. 4, *L*).

The level of expression of the gene for the GluA1 subunit of the AMPA receptor was not different the mPFC of in adult animals of the three groups (H = 3.2, $p = 0.21$, Kruskal–Wallis test, Fig. 5, *A*), while increased levels were seen in the dorsal and ventral areas of the hippocampus, these being statistically significant for the dorsal area after LPS 25 μg/kg (physiological saline/LPS25 $p < 0.01$, physiological saline/LPS50 $p = 0.08$, pairwise comparison, Dunn's test, Fig. 5, *B*) and after LPS 50 μg/kg in the ventral area (physiological saline/LPS25 $p = 0.83$, physiological saline/LPS50 $p < 0.01$, pairwise comparison, Dunn's test, Fig. 5, *C*).

A high level of mRNA for the GluA2 AMPA receptor subunit was also seen in the ventral area of the hippocampus, after 50 μg/kg LPS as compared with control animals (physiological saline/LPS25 $p = 0.80$, physiological saline/LPS50 $p = 0.03$, pairwise comparison, Dunn's test, Fig. 5, *F*). There were no differences in the level of expression of the *Gria2* gene in the mPFC and DH of adult animals of the three groups (H = 4.8, $p = 0.09$; H = 4.2, $p = 0.12$, Kruskal–Wallis test, (Fig. 5, *D*, *E*).

Discussion. The present studies identified long-term impairments to exploratory behavior, decreased anxiety levels, and changes in the expression of genes for the subunits of ionotropic glutamate receptors in the rat hippocampus after administration of bacterial LPS in the early postnatal period at doses inducing the development of inflammatory reactions (increased proinflammatory cytokine synthesis) in the brain.

The closest analog to our work is a study reported by Harré et al., [46], who assessed the expression of genes for

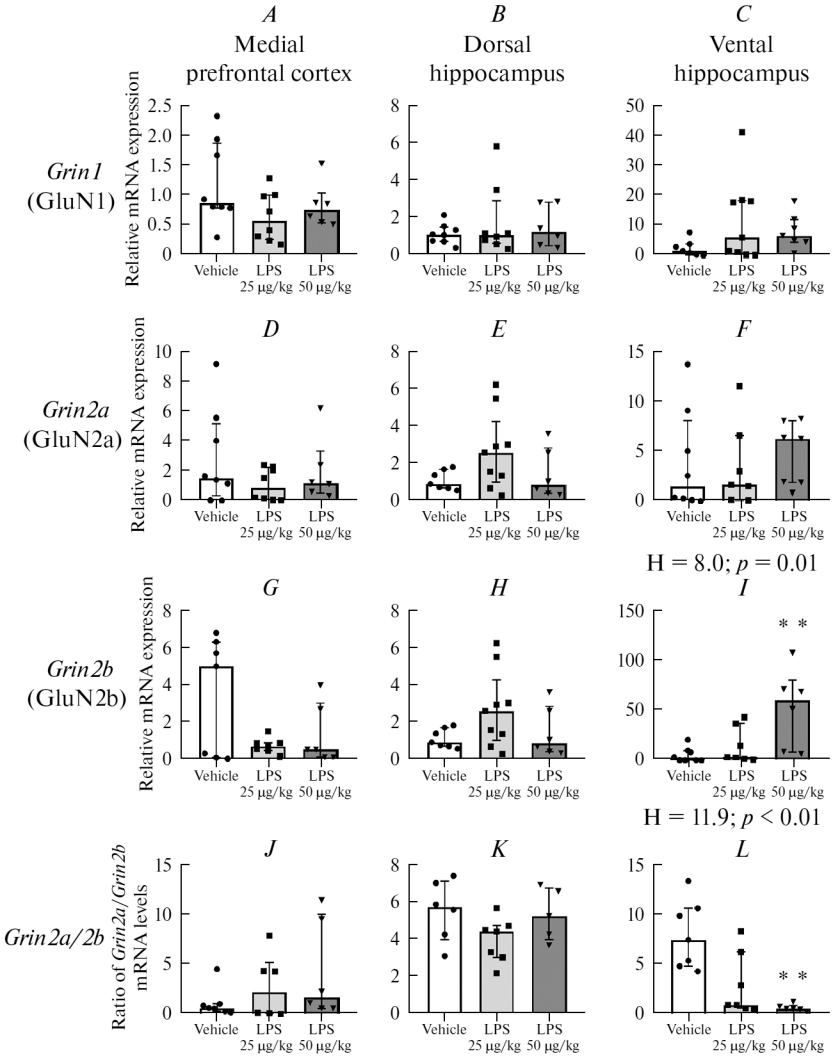

Fig. 4. Changes in the level of expression of genes for NMDA receptor subunits in the brains of adult rats due to LPS administration at early age. H – Significant between-group differences, Kruskal–Wallis test; $**p < 0.01$ compared with control group, a posteriori Dunn's test. $n = 5–9$. Data are presented as medians and interquartile intervals.

NMDA receptors in rats given single doses of bacterial LPS at different stages of early postnatal ontogeny. Features of our study were that apart from NMDA receptor subunits, we addressed changes in the expression of the genes for AMPA receptor subunits, LPS was given as courses, and each LPS dose was smaller (25 or 50 μg/kg vs. 100 μg/kg used by Harré et al.). In addition, our study included a differential analysis of changes occurring in the ventral and dorsal parts of the hippocampus. The latter was important in relation to appearance in recent years of studies demonstrating different functional roles for the ventral and dorsal areas of the hippocampus [47–49].

Our studies showed that courses of LPS in the third week of life increased the expression of the gene for the GluN2b subunit of the NMDA receptor and decreased the GluN2a/2b mRNA ratio in the ventral but not the dorsal hippocampus and not in the medial prefrontal cortex. These results are consistent with data obtained by Harré et al., who demonstrated an increase in the production of GluN2b mRNA in rat hippocampal cells after administration of LPS on day 14 of life [46]. In contrast to these authors, we did not find any increase in the expression of the GluN2b subunit gene in the frontal cortex, nor any increase in the expression of the GluN2a subunit gene in the hippocampus, which may be associated with the lower LPS dose used in our experiments.

Expression of AMPA receptor subunit genes was not studied in the work reported by Harré et al., though it was investigated by Galic et al. using another experimental model – after intracerebroventricular administration of PolyI:C (a synthetic molecule which models viral infection) on day 14 of life. In adulthood, these experimental rats showed increases in the expression of the GluA1 subunit gene in the hippocampus [50], which is consistent with our results obtained in

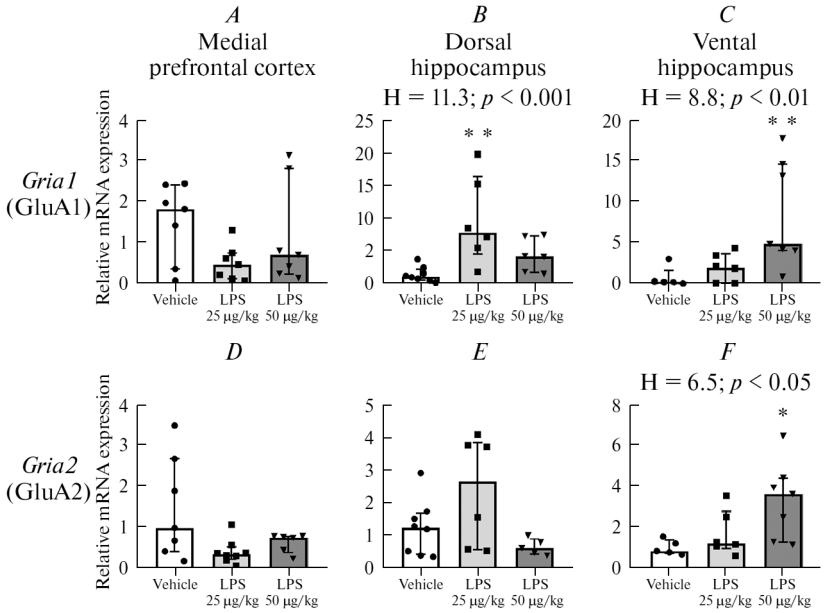

Fig. 5. Changes in the level of expression of genes for AMPA receptor subunits in the brains of adult rats due to LPS administration at early age. H – Significant between-group differences, Kruskal–Wallis test; *$p < 0.05$; **$p < 0.01$ compared with control group, a posteriori Dunn's test. n = 5–8. Data are presented as medians and interquartile intervals.

the bacterial LPS model. In addition, we found an increase in the production of GluA2 subunit mRNA in the ventral hippocampus, which was not seen in the PolyI:C model [50].

Attention is drawn to the fact that the most marked changes in the expression of genes both for proinflammatory cytokines and ionotropic glutamate receptor subunits were seen in these experiments in the hippocampus but not in the medial prefrontal cortex. The greater sensitivity to systemic LPS administration of hippocampal neurons over medial prefrontal cortex neurons has been demonstrated in electrophysiological studies [51]. Systemic LPS administration produces spike-wave discharges in the hippocampus but not the cortex. Some authors have suggested that the hippocampus has selectively elevated sensitivity to proinflammatory agents [47]. This may be linked with the high density of receptors for proinflammatory cytokine interleukin-1, which mediates the central effects of LPS, in hippocampal cells [52].

In our studies, adult rats given LPS in the third week of life showed more marked changes in the expression of genes for NMDA and AMPA receptor subunits in the ventral (as compared with the dorsal) hippocampus. As the ventral hippocampus plays an important role in regulating emotional behavior, particularly anxiety levels [47], these results provided the opportunity to analyze the effects of neonatal administration of LPS on anxiety behavior in adult animals.

Courses of LPS in week 3 of life were found to lead to minor decreases in exploratory behavior in the open field test and anxiety levels in the elevated plus maze.

The influence of neonatal injections of LPS on these forms of behavior have previously been evaluated in a number of studies. Most investigators gave injections of LPS to experimental animals in the first days of life. Increased anxiety, including in the elevated plus maze, was demonstrated in adolescent rats given injections of LPS 50 μg/kg on days 5 and 7 of life [53]. High anxiety levels were also seen in adult rats given LPS 25 μg/kg on the first day of life [54] or 50 μg/kg on days 3 and 5 of life [55]. However, other authors using the same models (administration of 50 μg/kg of LPS on days 3 and 5 of life) noted decreased anxiety in adolescents [56] and adults [57].

There are significantly fewer studies in which LPS was given in the third week of life. Doenni et al. showed that injection of LPS 100 μg/kg on day 14 of life led to impairment of the extinction of fear in response to a conditioned stimulus in adult trained rats, with no impairment to behavior in the elevated plus maze [58]. Spencer et al. used an analogous experimental paradigm and found no changes in anxiety levels, though impairments to exploratory behavior were demonstrated [59]. The effects of neonatal LPS injections on anxiety levels may depend on the endotoxin dose and its timing.

The decreased anxiety levels seen here may be associated with the changes seen in the expression of NMDA receptor genes in the ventral hippocampus. Involvement of these receptors in controlling anxiety behavior has previously been demonstrated in several pharmacological studies. Thus, administration of N-methyl-D-aspartate itself into the ventral hippocampus was found to increase the time spent in the open arms of the elevated plus maze, which is evidence of decreased anxiety [60]. Furthermore, injections of the NMDA receptor antagonist D-AP5 into the same part of the brain blocked the anxiolytic effect of morphine [61].

Overall, our studies showed that administration of bacterial LPS during the period of active formation of NMDA and AMPA glutamate receptors led to long-term changes in the expression of the genes for the subunits of these receptors and their associated forms of behavior.

This study was supported by the Russian Foundation for Basic Research (Grant No. 17-04-02116) and Program No. 18 of the Presidium of the Russian Academy of Sciences.

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