

Early and Delayed Changes in Neuroplasticity-Related Gene Expression after Postnatal Interleukin-1 β and Lipopolysaccharide Administration in Rats

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Research Article

Keywords: early postnatal development, interleukin-1 β , lipopolysaccharide, neuroplasticity, Fgf2, Mmp9/Timp1

Posted Date: October 14th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11152567/v1>

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Additional Declarations: No competing interests reported.

Abstract

Inflammatory challenges during early postnatal development may alter brain maturation and contribute to later cognitive impairment. This study compared early and delayed changes in neuroplasticity-related gene expression after systemic interleukin-1 β (IL-1 β) or lipopolysaccharide (LPS) administration. Fifty-four male Wistar rats were included. IL-1 β was administered intraperitoneally at 1 μ g/kg daily on postnatal days 15–21, whereas LPS was administered at 25 μ g/kg on days 15, 18, and 21. *Bdnf*, *Fgf2*, *Mmp9*, and *Timp1* mRNA levels were measured by reverse transcription quantitative polymerase chain reaction in the medial prefrontal cortex (mPFC) and dorsal hippocampus (DH), 2 h after the final injection and at 2.5 months of age. Reference genes were selected from nine candidates separately for each age and brain region. After IL-1 β treatment, *Fgf2* mRNA was elevated in the mPFC at the early time point. Adult LPS-exposed rats had lower *Fgf2* mRNA in the mPFC and a lower ratio of relative *Mmp9* to *Timp1* mRNA levels in the DH. *Fgf2* mRNA in the adult DH changed in the same direction, but the comparison with controls was at the significance threshold ($p = 0.0500$). No significant differences in total *Bdnf* mRNA were detected. The two treatment regimens produced different molecular response profiles, whose functional significance requires further investigation.

1. Introduction

Normal brain development is essential for the formation of cognitive functions (Stiles and Jernigan 2010). Maturation of the central nervous system (CNS) begins during embryonic development and continues after birth, involving neuronal and glial differentiation, synaptogenesis, myelination, and remodeling of intercellular interactions (Rice and Barone 2000). Inflammation during critical developmental periods may alter subsequent brain maturation. In experimental studies, early bacterial infection or lipopolysaccharide (LPS) administration has been associated with later changes in behavior, synaptic plasticity, and responses to subsequent immune challenge or learning (Bilbo et al. 2005, 2008; Wang et al. 2013; Tishkina et al. 2016). Administration of interleukin-1 β (IL-1 β) during the third postnatal week impaired spatial memory and active and passive avoidance learning in adult rats (Zubareva et al. 2013; Trofimov et al. 2014).

Systemic LPS administration is widely used to model early-life inflammation. It activates innate immune responses and the production of proinflammatory mediators, including IL-1 β . Direct IL-1 β administration allows the effects of elevated levels of this cytokine, considered a major mediator of inflammation in the developing brain, to be examined. Involvement of IL-1-dependent mechanisms is supported by the attenuation of delayed impairments by an IL-1 receptor antagonist and, in another early LPS exposure model, by suppression of IL-1 β expression (Lan et al. 2015; Zhang et al. 2022). However, LPS activates a broader range of cellular responses, and its effects cannot be attributed to the elevation of a single cytokine (Mittli et al. 2023). Previous studies found impaired learning in adult rats after either IL-1 β or LPS administration during the third postnatal week (Trofimov et al. 2014, 2017). Similar behavioral outcomes do not, however, establish that the underlying molecular changes are the same.

Altered expression of genes involved in neuroplasticity is one possible mechanism underlying these impairments. The dorsal hippocampus (DH), which contributes to spatial learning, and the medial prefrontal cortex (mPFC), which participates in memory consolidation and the use of acquired information, are of particular interest (Euston et al. 2012; Yadav et al. 2024; Rolls and Treves 2024). Both regions belong to distributed memory systems, with hippocampal–entorhinal interactions also contributing to spatial memory (Steffenach et al. 2005; Rolls and Treves 2024). Relevant molecular factors include brain-derived neurotrophic factor (BDNF), fibroblast growth factor-2 (FGF-2), matrix metalloproteinase-9 (MMP-9), and tissue inhibitor of metalloproteinases-1 (TIMP-1). BDNF and FGF-2 participate in neurogenesis and synaptic plasticity (Yoshimura et al. 2001; Vaynman et al. 2004; Zechel et al. 2010; Toader et al. 2025; Wang et al. 2025). The MMP-9/TIMP-1 system regulates extracellular proteolysis around synapses, including the modification of cell adhesion proteins. Extracellular matrix remodeling is also associated with structural plasticity and memory (Jourquin et al. 2005; Chaillan et al. 2006; Vafadari et al. 2016; Wiera and Mozrzymas 2021; Sanchez et al. 2024; Ganguly et al. 2024).

Disruption of fibroblast growth factor receptor (FGFR) signaling is associated with altered neurogenesis, long-term potentiation (LTP), and memory consolidation. FGFR1/2 signaling in oligodendroglial cells also contributes to synaptic function (Zhao et al. 2007; Furusho et al. 2026). LTP induction and maintenance depend on coordinated regulation of MMP-9 activity and its inhibitors (Nagy et al. 2006; Okulski et al. 2007; Wiera and Mozrzymas 2021). Conversely, increasing *Fgf2* expression through gene transfer improved cognition and hippocampal synaptic function in the transgenic Alzheimer's disease models examined (Kiyota et al. 2011). These findings support investigation of these molecular systems after early inflammatory exposure, without implying a direct correspondence between an individual mRNA level and memory function.

We previously found lower *Fgf2* mRNA levels in the mPFC and ventral hippocampus of adult rats exposed to IL-1 β during the third postnatal week after active avoidance training, but not in untrained animals (Trofimov et al. 2016). Following early LPS administration, *Mmp9* and *Timp1* mRNA levels also differed according to brain region, age, and previous training (Trofimov et al. 2017). An independent study demonstrated altered learning-induced *Bdnf* expression after early infection (Bilbo et al. 2008). However, the extent to which IL-1 β and LPS regimens produce similar early and delayed changes in these markers in the same brain regions of untrained animals remains unclear.

Measurement of mRNA levels by reverse transcription quantitative polymerase chain reaction (RT-qPCR) requires appropriate internal controls. Reference gene suitability must be assessed under the experimental conditions, because expression may vary with age, tissue, or treatment (Vandesompele et al. 2002; Andersen et al. 2004; Bustin et al. 2009). A previously developed multiplex assay permits the assessment of nine candidate reference genes in three triplex reactions (Schwarz et al. 2020). Normalization to several selected reference genes reduces dependence on the variability of a single gene (Vandesompele et al. 2002).

This study compared early and delayed changes in *Bdnf*, *Fgf2*, *Mmp9*, and *Timp1* mRNA levels, and the ratio of relative *Mmp9* to *Timp1* mRNA levels, in the mPFC and DH after moderately pyrogenic IL-1 β or LPS treatment during the third postnatal week. Analyses were performed 2 h after the final injection and at 2.5 months of age in rats that had not undergone behavioral testing. Reference genes were selected from nine candidates separately for each age and brain region.

2. Materials and Methods

2.1. Experimental Animals

The study included 54 male Wistar rats (*Rattus norvegicus*). Animals were housed in plastic cages (55 × 30 × 15 cm) at 22–24°C under a 12-h light/dark cycle (lights on at 07:00 and off at 19:00). Procedures were performed after 19:00. Food and water were available *ad libitum*. Pups were housed with the dam, and litter sizes were standardized after birth. All experimental procedures were approved by the Local Ethics Committee of the Institute of Experimental Medicine, Saint Petersburg, Russia (Protocol No. 4/25, 25 December 2025), and were conducted in accordance with Council Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes.

2.2. Experimental Design

On postnatal day 15 (P15), rats were assigned to four groups. IL-1 β was administered intraperitoneally at 1 μ g/kg daily from P15 to P21, for a total of seven injections ($n = 15$). LPS (serotype O111:B4; Sigma-Aldrich) was administered at 25 μ g/kg on P15, P18, and P21, for a total of three injections ($n = 12$). Corresponding control groups received pyrogen-free saline on the seven-injection ($n = 15$) or three-injection ($n = 12$) schedule (Fig. 1). The injection volume was 0.1 mL per 10 g body weight.

Doses and schedules were selected from previous studies using these models (Trofimov et al. 2014, 2017). Intervals between LPS injections were used to limit the development of tolerance to repeated endotoxin exposure.

To assess early effects, 28 rats were decapitated 2 h after the final injection on P21: 7 IL-1 β controls, 8 IL-1 β -treated rats, 7 LPS controls, and 6 LPS-treated rats. The remaining animals were maintained until 2.5 months of age without behavioral testing and were individually housed from 1.5 months of age. The adult cohort comprised 26 rats: 8 IL-1 β controls, 7 IL-1 β -treated rats, 5 LPS controls, and 6 LPS-treated rats.

2.3. mRNA Level Analysis

After decapitation, brains were removed, immediately frozen, and stored at – 70°C. The mPFC and DH were dissected from coronal sections using a MICROM HM 525 cryostat (Thermo Fisher Scientific, USA) at – 20°C. Regional boundaries were identified using the atlas of Paxinos and Watson (Paxinos and Watson 2006), whereas the age-specific stereotaxic atlas of Khazipov et al. (Khazipov et al. 2015) was used for P21 brains (Fig. 2).

Total RNA was isolated by guanidinium thiocyanate–phenol–chloroform extraction using ExtractRNA (Evrogen, Russia). Following phase separation, RNA was precipitated with isopropanol and washed with 75% ethanol. Genomic DNA was removed using RQ1 DNase (Promega, USA), followed by RNA reprecipitation with lithium chloride. RNA concentration and the absorbance ratio at 260 and 280 nm were measured using a NanoDrop 2000 (Thermo Fisher Scientific, USA).

Reverse transcription was performed in 20 μ L using 2 μ g RNA, a mixture of oligo(dT) primers and random nonamers (DNA Synthesis, Russia), and 200 U M-MLV reverse transcriptase (Promega, USA). Reactions were incubated at 42°C for 60 min, followed by enzyme inactivation at 65°C for 10 min. The resulting cDNA was diluted fivefold with water and stored at – 20°C.

Quantitative PCR was performed with TaqMan hydrolysis probes on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, USA). Candidate reference genes were analyzed in three triplexes: *Actb/Gapdh/B2m*, *Rpl13a/Sdha/Ppia*, and *Hprt1/Pgk1/Ywhaz* (Schwarz et al. 2020). *Fgf2* and *Bdnf* were measured in a duplex assay, whereas *Mmp9* and *Timp1* were analyzed in separate singleplex reactions. The *Bdnf* assay targeted the shared coding exon. Primer and probe sequences are provided in Table 1.

Each 10- μ L reaction contained 1 μ L 10 $\times$ buffer, 1.4 μ L 25 mM MgCl₂, 0.1 μ L dNTP mix (25 mM each), 0.2 μ L primer/probe mix for each target, 1 μ L cDNA, and 0.15 μ L TaqM DNA polymerase (5 U/ μ L; Alkor Bio, Russia), with water added to the final volume. Each primer/probe mix contained 10 μ L of each primer (20 pmol/ μ L) and 10 μ L probe (5 pmol/ μ L), made up to 50 μ L with water. Cycling conditions were 95°C for 10 min; four cycles of 95°C for 5 s, 60°C for 10 s, and 72°C for 15 s; and 50 further cycles under the same conditions, with fluorescence recorded during extension.

Samples were analyzed in technical triplicate. No-template controls contained water instead of cDNA. Groups compared directly within an analysis were included in the same run. Quantification cycle (Cq) values were determined by the threshold method in CFX Manager 3.1 and averaged across technical replicates. Amplification efficiency and the coefficient of determination of the standard curve were assessed using serial fourfold cDNA dilutions (**Supplementary Fig. S1**).

Expression stability of the nine candidate reference genes was evaluated separately for the mPFC and DH at each age, with all treatment groups included in the corresponding sample set. RefFinder was used to integrate rankings from geNorm, NormFinder, BestKeeper, and the comparative Δ Cq approach (Vandesompele et al. 2002; Pfaffl et al. 2004; Andersen et al. 2004; Silver et al. 2006). The two genes with the best overall ranks were

selected for normalization. Relative *Bdnf*, *Fgf2*, *Mmp9*, and *Timp1* mRNA levels were calculated using the $E^{-\Delta\Delta Cq}$ method (Livak and Schmittgen 2001). ΔCq was calculated by subtracting the arithmetic mean Cq of the two reference genes from the target gene Cq ($\Delta Cq(g,k) = Cq(g,k) - [Cq(r1,k) + Cq(r2,k)] / 2$); $\Delta\Delta Cq$ was then calculated relative to the mean control ΔCq ($\Delta\Delta Cq(g,k) = \Delta Cq(g,k) - \text{mean } \Delta Cq(g,\text{control})$) (Livak and Schmittgen 2001; Vandesompele et al. 2002). The ratio of relative *Mmp9* to *Timp1* mRNA levels was also analyzed.

Table 1
Primer and hydrolysis probe sequences used for RT-qPCR.

Reaction	Gene, RefSeq	Forward primer, 5'→3'	Reverse primer, 5'→3'	Probe, 5'→3'
Multiplex 1	<i>Actb</i> , NM_031144	TGTCACCAACTGGGACGATA	GGGGTGTGAAGGTCTCAAA	CGTGTGGCCCCTGAGGAGCAC
	<i>Gapdh</i> , NM_017008	TGCACCACCAACTGCTTAG	GGATGCAGGGATGATGTTC	ATCACGCCACAGCTTTCCAGAGGG
	<i>B2m</i> , NM_012512	TGCCATTTCAGAAACTCCCC	GAGGAAGTTGGGCTTCCCATT	ATTCAAGTGTACTCTCGCCATCCACCG
Multiplex 2	<i>Rpl13a</i> , NM_173340	GGATCCCTCCACCCTATGACA	CTGGTACTTCCACCCGACCTC	CTGCCCTCAAGGTTGTGCGGCT
	<i>Sdha</i> , NM_130428	AGACGTTTGACAGGGGAATG	TCATCAATCCGCACCTTGTA	ACCTGGTGGAGACGCTGGAGCT
	<i>Ppia</i> , NM_017101	AGGATTCATGTGCCAGGGTG	CTCAGTCTTGGCAGTGCAGA	CACGCCATAATGGCACTGGTGGCA
Multiplex 3	<i>Hprt1</i> , NM_012583	TCCTCAGACCGCTTTTCCCGC	TCATCATCACTAATCACGACGCTGG	CCGACCGGTTCTGTCATGTCGACCCT
	<i>Pgk1</i> , NM_053291	ATGCAAAGACTGGCCAAGCTAC	AGCCACAGCCTCAGCATATTTT	TGCTGGCTGGATGGGCTTGGA
	<i>Ywhaz</i> , NM_013011	GATGAAGCCATTGCTGAACTTG	GTCTCCTTGGGTATCCGATGTC	TGAAGAGTCGTACAAAGACAGCACGC
Multiplex 4	<i>Fgf2</i> , NM_019305	AGCGGCTCTACTGCAAGAAC	TGGAGCTGTAGTTTGACGTGT	AGACGGCCGCGTGGACGGCGTCCG
	<i>Bdnf</i> , NM_001270631	CCATAAGGACGCGGACTTGAC	GAGGAGGCTCCAAAGGCACTT	CTTCCCGGGTGATGCTCAGCAGT
Singleplex	<i>Mmp9</i> , NM_031055	CCTCTGCATGAAGACGACAT	GAGGTGCAGTGGGACACATA	CTGTATGGTCGTGGCTCTAAACCTGA
Singleplex	<i>Timp1</i> , NM_053819	CTGGCATAATCTGAGCCCTG	GCAAAGTGATCGCTCTGGTAG	TGTGCACAGTGTTTCCCTGTTTCAGC

2.4. Statistical Analysis

Statistical analyses were performed in GraphPad Prism 8 (GraphPad Software, USA) separately for each age and brain region. Technical replicates were averaged before between-group analyses. Normality was assessed using the Shapiro–Wilk test. The two control groups were compared using Student’s *t* test, Welch’s *t* test, or the Mann–Whitney *U* test, depending on the distributions and variances. In the reported analysis, the control groups were pooled after no statistically significant differences were detected between them. When parametric assumptions were met, the three groups were compared using one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparisons test. The *Mmp9/Timp1* ratio in the adult DH was analyzed using the Kruskal–Wallis test followed by Dunn’s multiple comparisons test. When variances were unequal, Brown–Forsythe and Welch ANOVA with Dunnett’s T3 multiple comparisons test was used. Data are presented as mean ± standard error of the mean (SEM). The significance threshold was $p < 0.05$.

3. Results

3.1. Amplification Characteristics of the Primer–Probe Sets

Amplification efficiencies in the three reference-gene triplex assays ranged from 95.1 to 109.3%: *Actb*, 100.3%; *Gapdh*, 98.5%; *B2m*, 102.6%; *Rpl13a*, 96.6%; *Sdha*, 109.3%; *Ppia*, 99.8%; *Hprt1*, 102.5%; *Pgk1*, 96.5%; and *Ywhaz*, 95.1% (**Supplementary Fig. S1**).

In the *Fgf2/Bdnf* duplex assay, amplification efficiencies were 101.4% for *Fgf2* and 99.7% for *Bdnf*, with R^2 values of 0.992 and 0.988, respectively. Co-amplification of *Mmp9* and *Timp1* did not yield data suitable for further analysis, so these genes were analyzed in separate singleplex reactions. *Mmp9* amplification efficiency was 105.0% with $R^2 = 0.929$; the corresponding values for *Timp1* were 102.5% and $R^2 = 0.891$.

3.2. Reference Gene Selection

The overall RefFinder rankings differed across the four sample sets (**Fig. 3**). *Pgk1* and *B2m* ranked as the most stable genes in the mPFC at P21, whereas *Hprt1* and *B2m* ranked highest in the adult mPFC. In the DH, the highest-ranked genes were *B2m* and *Rpl13a* at P21 and *Pgk1* and *Ppia* in adults. These pairs were used for normalization. *Actb* and *Gapdh* ranked among the least stable genes in all four sample sets. No single pair ranked highest across all age–region combinations.

3.3. Effects of IL-1 β and LPS on Neuroplasticity-Related mRNA Levels

After pooling the two control groups, which did not differ significantly, control, IL-1 β , and LPS groups were compared separately for each brain region and age (**Fig. 4**). At P21, 2 h after the final injection, *Fgf2* mRNA levels differed between groups in the mPFC (ANOVA: $F(2, 11) = 12.00, p = 0.0017$). Levels were higher in the IL-1 β group than in the pooled control group (Tukey's test: $p = 0.0015$) and the LPS group ($p = 0.0131$). No significant difference was detected between LPS-treated rats and controls. Total *Bdnf* mRNA and the *Mmp9/Timp1* ratio did not differ significantly between groups in the mPFC at P21.

In the DH at P21, no significant between-group differences were detected in *Bdnf* or *Fgf2* mRNA levels or the *Mmp9/Timp1* ratio. Thus, among the markers assessed at the early time point, significant differences were detected for *Fgf2* in the mPFC.

In adult rats, mPFC *Fgf2* mRNA levels differed between groups (ANOVA: $F(2, 19) = 10.22, p = 0.0010$). Levels were lower in the LPS group than in controls (Tukey's test: $p = 0.0151$) and the IL-1 β group ($p = 0.0008$). No significant difference was detected between IL-1 β -treated rats and controls. Total *Bdnf* mRNA and the *Mmp9/Timp1* ratio did not differ significantly between groups in the adult mPFC.

In the adult DH, *Fgf2* mRNA levels differed between groups (ANOVA: $F(2, 20) = 4.764, p = 0.0203$). Levels were lower in the LPS group than in the IL-1 β group (Tukey's test: $p = 0.0167$). The mean was also lower in the LPS group than in controls, although this comparison was at the stated significance threshold (Tukey's test: $p = 0.0500$).

The *Mmp9/Timp1* ratio also differed between groups in the adult DH (Kruskal–Wallis test: $H = 11.46, p = 0.0032$). The ratio was lower in the LPS group than in controls (Dunn's test: $p = 0.0028$) and the IL-1 β group ($p = 0.0233$). No significant difference was detected between IL-1 β -treated rats and controls. Total *Bdnf* mRNA did not differ significantly between groups in the adult DH.

4. Discussion

The IL-1 β and LPS regimens used during the third postnatal week produced different molecular response profiles. After IL-1 β treatment, *Fgf2* mRNA was elevated in the mPFC at P21. Adult rats exposed to LPS had lower *Fgf2* mRNA in the mPFC and a lower *Mmp9/Timp1* ratio in the DH. *Fgf2* mRNA in the DH changed in the same direction, although the comparison with controls was borderline. No significant differences in total *Bdnf* mRNA were detected. The observed changes therefore differed between the two early inflammatory exposure regimens.

Differences in the inflammatory response may partly explain these findings. LPS acts through Toll-like receptor 4 (TLR4) and induces a broad response that includes IL-1 β and other mediators. IL-1 β administration can also alter cytokine production secondarily, so its effects cannot be considered an isolated action of one molecule (Mittli et al. 2023). The contribution of IL-1-dependent mechanisms is supported by attenuation of the delayed effects of early LPS exposure with an IL-1 receptor antagonist (Lan et al. 2015). In a severe inflammation model involving LPS administration at P3, suppression of IL-1 β expression attenuated cognitive impairment and abnormalities in GABAergic maturation associated with potassium–chloride cotransporter 2 (KCC2) regulation (Zhang et al. 2022). However, that model differs from ours in the timing and intensity of exposure. Our comparison also involved seven IL-1 β injections versus three LPS injections. The observed differences therefore apply to the complete regimens and do not separate the properties of the inflammatory agents from injection frequency.

The early increase in mPFC *Fgf2* mRNA after IL-1 β treatment may reflect a response of developing tissue to cytokine exposure. FGF-2 participates in precursor-cell proliferation, neurogenesis, and recovery after injury (Yoshimura et al. 2001; Zechel et al. 2010; Wang et al. 2025). Prenatal and postnatal FGF-2 administration altered cortical morphology and facilitated recovery after early mPFC injury (Comeau et al. 2007). However, increased mRNA alone does not establish a neuroprotective response. It could reflect compensatory processes, changes in glial state, or altered tissue cellular composition. Because total tissue RNA was analyzed, the cellular source and functional significance of the increase remain unresolved.

Adult rats exposed to IL-1 β showed no significant difference in basal *Fgf2* mRNA compared with controls. A similar result was obtained in our earlier study in untrained animals. After active avoidance training, however, rats exposed to IL-1 β early in life had lower *Fgf2* mRNA in the mPFC and ventral hippocampus than controls (Trofimov et al. 2016). Absence of a basal difference therefore does not exclude an altered response to subsequent challenge. A comparable learning-dependent molecular response has been described for *Bdnf* after early infection (Bilbo et al. 2008). This reactivity was not examined here, and any impairment of it remains hypothetical.

Early LPS exposure was followed by lower *Fgf2* mRNA in the adult mPFC. Mean values changed in the same direction in the DH, but the difference from controls was at the significance threshold. These observations suggest a delayed alteration in *Fgf2* regulation. FGF-2 deficiency alters hippocampal dendritic spine morphology (Zechel et al. 2009), whereas disruption of FGFR1 signaling affects neurogenesis, LTP, and memory consolidation (Zhao et al. 2007). More recent evidence also implicates oligodendroglial FGFR1/2 signaling in synaptic function and memory (Furusho

et al. 2026). However, receptor interventions affect responses to several FGF ligands and are not equivalent to a reduction in *Fgf2* mRNA. Attributing this transcriptional finding to a specific cellular mechanism would therefore be premature.

The potential for functional modification of this system has been demonstrated by hippocampal *Fgf2* gene transfer in transgenic Alzheimer's disease models. The intervention improved memory in one model and restored hippocampal synaptic function in another transgenic line (Kiyota et al. 2011). These findings support investigation of FGF-2 as a plasticity factor, but do not establish that its supplementation would reverse the effects of early inflammation. We did not measure FGF-2 protein, receptor activation, neurogenesis, or behavior in the same animals. The relationship between reduced *Fgf2* mRNA and previously reported learning impairments after LPS thus remains hypothetical (Tishkina et al. 2016; Trofimov et al. 2017).

The lower *Mmp9/Timp1* ratio in the adult DH involves another functionally related neuroplasticity system. MMP-9 contributes to modification of the synaptic environment, late-phase LTP, and long-term memory (Nagy et al. 2006, 2007). Local translation of *Mmp9* mRNA is regulated by neuronal activity (Dziembowska et al. 2012). TIMP-1 limits metalloproteinase activity, and changes in its abundance affect neuronal survival, axonal plasticity, and learning (Jourquin et al. 2005; Chaillan et al. 2006). Synaptic function requires spatially and temporally regulated proteolysis; more MMP-9 does not invariably improve plasticity (Okulski et al. 2007; Vafadari et al. 2016; Wiera and Mozrzymas 2021). These processes are part of the broader remodeling of extracellular matrix and perineuronal nets during learning (Sanchez et al. 2024; Ganguly et al. 2024).

The ratio of relative *Mmp9* to *Timp1* mRNA levels describes only the relationship between two transcriptional measures. A lower ratio may result from reduced *Mmp9*, increased *Timp1*, or both, and the ratio alone cannot distinguish these possibilities. It does not measure stoichiometric binding between the protease and its inhibitor. MMP-9 activity additionally depends on translation, secretion and activation of pro-MMP-9, interaction with inhibitors, and substrate availability (Dziembowska et al. 2012; Vafadari et al. 2016; Wiera and Mozrzymas 2021). Our data therefore do not establish reduced proteolytic activity in the DH. Such an interpretation requires separate *Mmp9* and *Timp1* measurements together with protein abundance and activity assays.

No significant differences in total *Bdnf* mRNA were detected in any sample set. This negative finding is restricted to the sampling times and assay sensitivity and does not exclude BDNF-dependent mechanisms. Individual *Bdnf* transcripts may respond differently to an intervention (Nair et al. 2007), whereas an assay targeting the shared coding region cannot resolve these responses. BDNF abundance and action also depend on translation, secretion, and protein processing (Toader et al. 2025). In an early-infection study, basal *Bdnf* mRNA did not differ, but learning-induced hippocampal *Bdnf* expression was attenuated in animals infected early in life (Bilbo et al. 2008). Absence of a basal difference is therefore compatible with altered system reactivity, although this possibility was not tested in our cohort.

Reference gene selection also requires consideration. The most stable pairs differed between sample sets, whereas *Actb* and *Gapdh* ranked poorly, supporting experimental validation of internal controls (Vandesompele et al. 2002; Bustin et al. 2009; Schwarz et al. 2020). Early and delayed measurements were obtained from independent cohorts, the two control schedules differed in injection frequency, and inflammatory response intensity was not measured. The findings therefore cannot be assigned unambiguously to specific signaling pathways. Only males were studied, and bulk-tissue mRNA does not identify the cellular source of a change or establish protein abundance and activity. Individual *Bdnf* transcripts, FGF-2 isoforms, MMP-9 activity, and behavior were not assessed. Within these limits, IL-1 β exposure was associated with an early increase in mPFC *Fgf2* mRNA, whereas LPS exposure was followed by lower adult mPFC *Fgf2* mRNA and a lower DH *Mmp9/Timp1* ratio. The adult DH *Fgf2* comparison with controls was borderline, and no significant differences in total *Bdnf* mRNA were detected. The functional significance of these molecular profiles requires validation at the protein, cellular, and behavioral levels.

Declarations

Acknowledgements

Not applicable.

Author Contributions

(I) Conception and design: Alexander N. Trofimov, Veronika A. Nikitina; (II) Administrative support: Alexander N. Trofimov; (III) Collection and assembly of data: Egor A. Shirokov; (IV) Data analysis and interpretation: Egor A. Shirokov, Alexander N. Trofimov; (V) Methodology and experimental supervision: Veronika A. Nikitina, Alexander N. Trofimov; (VI) Visualization: Egor A. Shirokov, Elizaveta A. Kim; (VII) Manuscript writing: All authors; (VIII) Final approval of manuscript: All authors.

Funding

This study was supported by the Ministry of Science and Higher Education of the Russian Federation within the state assignment of the Institute of Experimental Medicine, fundamental research project FGWG-2025-0018.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval

All experimental procedures were approved by the Local Ethics Committee of the Institute of Experimental Medicine, Saint Petersburg, Russia (Protocol No. 4/25, 25 December 2025), and were conducted in accordance with Council Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes.

Consent to Participate

Not applicable.

Consent to Publish

Not applicable.

Competing Interests

The authors declare no competing interests

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Figures

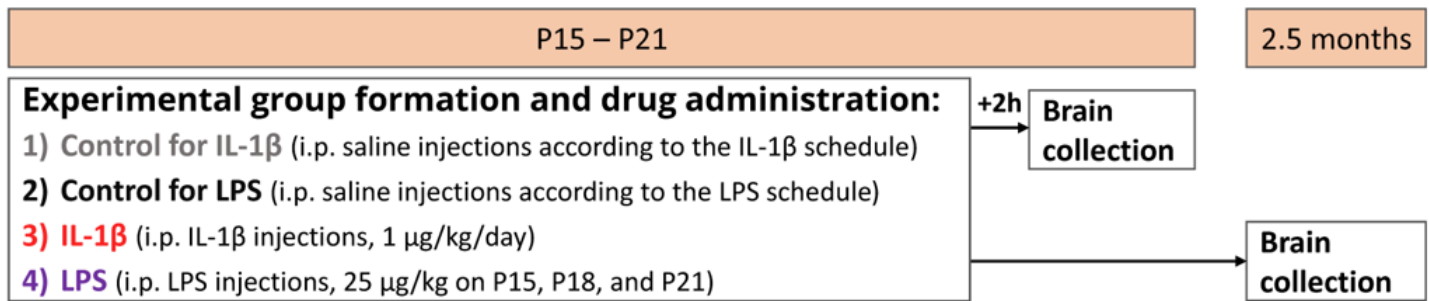


Figure 1

Experimental design. IL-1 β was administered daily from P15 to P21, and LPS on P15, P18, and P21. Control animals received saline according to the corresponding schedule. Brain tissue for subsequent molecular analysis was collected 2 h after the final injection or at 2.5 months of age.

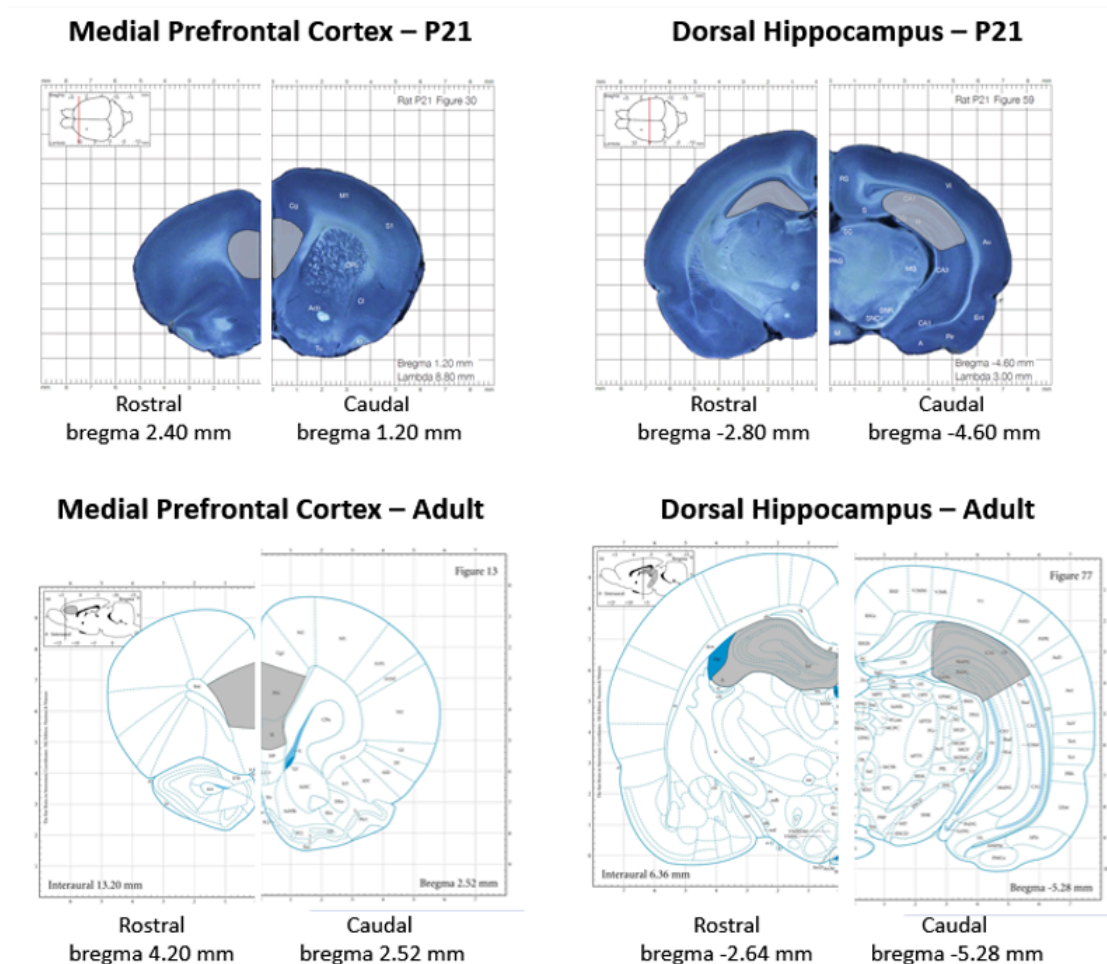


Figure 2

Dissection boundaries of the medial prefrontal cortex and dorsal hippocampus in P21 and adult rats. Shaded areas indicate the dissected tissue regions. P21 coronal sections were adapted from Khazipov et al. (Khazipov et al. 2015); adult sections were adapted from the atlas of Paxinos and Watson (Paxinos and Watson 2006).

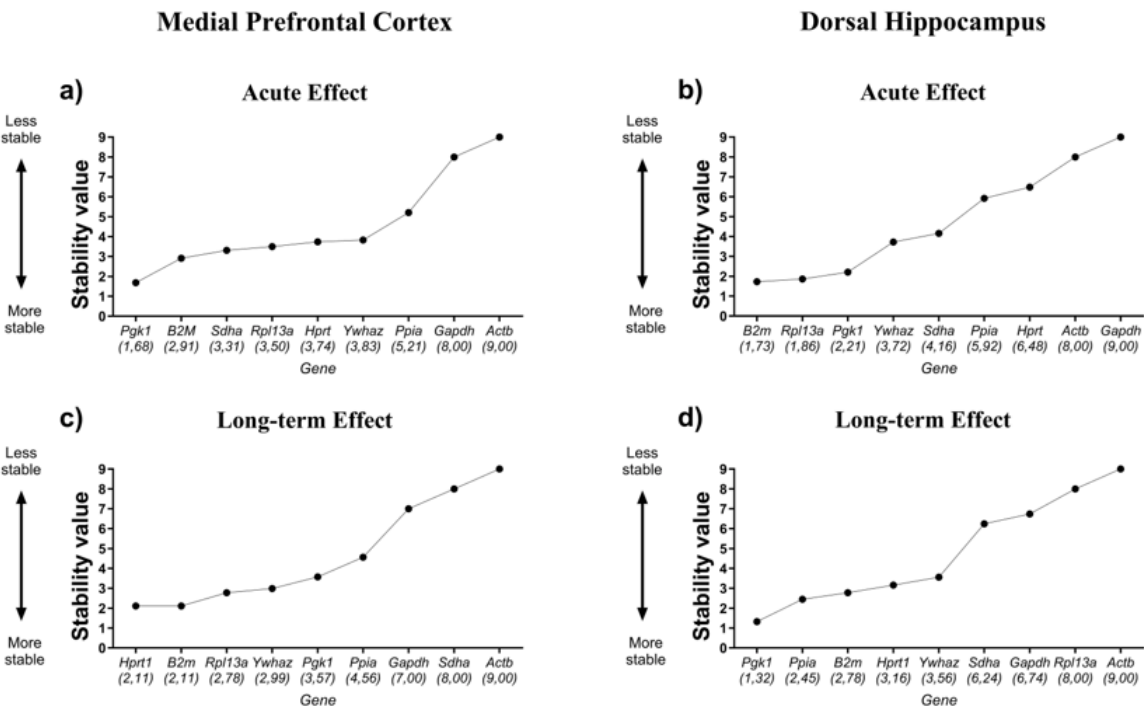


Figure 3

Ranking of candidate reference genes by age and brain region: **a** mPFC at P21; **b** DH at P21; **c** adult mPFC; **d** adult DH.

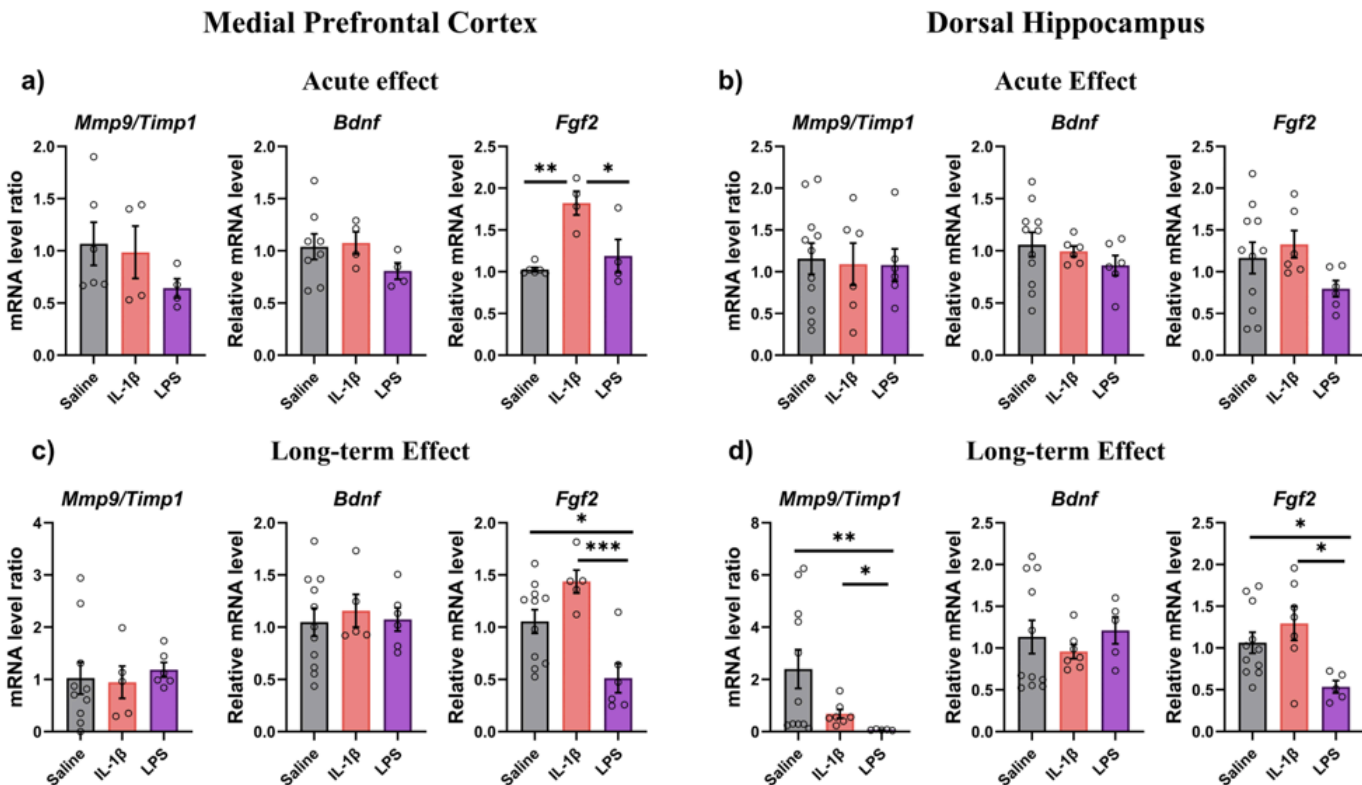


Figure 4

Relative *Fgf2* and *Bdnf* mRNA levels and the ratio of relative *Mmp9* to *Timp1* mRNA levels following IL-1 β or LPS administration: **a** mPFC at P21; **b** DH at P21; **c** adult mPFC; **d** adult DH. Data are presented as mean $\pm$ SEM.

Supplementary Files

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