Archival Report

Biological Psychiatry: GOS

Regional Molecular Changes in Chronic Lipopolysaccharide-Induced Neuroinflammation

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ABSTRACT

BACKGROUND: Neuroinflammation is linked to the development of depression. Exposure to the proinflammatory endotoxin lipopolysaccharide (LPS) is associated with a depressive-like phenotype in rodents. However, acute LPS exposure may reflect sickness behavior, and thus the molecular mechanisms and neurobehavioral changes associated with chronic neuroinflammation warrant investigation.

METHODS: Using male Sprague Dawley rats ($N = 37$) we investigated the impact of systemic inflammation, following a single or multiple doses of LPS on neurobehavioral outcomes and brain regional gene expression of inflammatory, neurotrophic and apoptotic markers in the prefrontal cortex, striatum, hippocampus, hypothalamus, midbrain, cortex, and cerebellum.

RESULTS: LPS administration induced systemic inflammation and subsequent neuroinflammation, as evidenced by increased circulating concentrations and regional expression of proinflammatory cytokines (*Tnf-α* and *Il1β*) in both short-term (ST) and long-term (LT) groups. Single LPS administration reduced the time spent in the center of the open field test after one week, while sucrose consumption was reduced with repeated LPS exposure. LPS showed a time- and region-specific effect on the expression of neurotrophins, as evidenced by increased messenger RNA expression of *Ngf* and *Nt-3* in both the ST-LPS and LT-LPS groups, while *Bdnf* and *Il6* expression was increased only in the LT-LPS group, and *Creb* expression was increased only in the ST-LPS group.

CONCLUSIONS: Taken together, our findings suggest that in LPS-induced systemic inflammation, *Tnf-α* and *Il1β* drive region-specific neurodegeneration via apoptotic processes, while *Il6* and its regulatory interaction with neurotrophins may serve as a protective mechanism in neuroinflammation.

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Neuroinflammation contributes to the development of neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis, and Huntington's disease (1–6). The region specificity of neuroinflammation often dictates the clinical symptoms of the disease, such as nigrostriatal dopaminergic neurodegeneration in PD and temporal degeneration in AD (7,8). Disorders such as depression (6) are linked to neuroinflammation across multiple brain regions, including the hippocampus (Hip), prefrontal cortex (Pfc), hypothalamus (Hyp), midbrain (Mdb), and striatum (Str) (9). Many of these disorders are preceded by chronic systemic inflammation, which increases blood-brain barrier (BBB) permeability, allowing peripheral cytokines to enter the brain and activate the brain's local immune responses (10,11). While acute neuroinflammation may have protective effects, chronic neuroinflammation upregulates the expression of proinflammatory cytokines, leading to synaptic degeneration and neuronal death (12–15). However, the various molecular mechanisms whereby neuroinflammation contributes to neuronal damage are currently under investigation.

The lipopolysaccharide (LPS) model, which has been widely used to study systemic inflammation–induced neuroinflammation, activates the nuclear factor-κB (NF-κB) pathway via toll-like receptor 4 (TLR4) which results in increased proinflammatory cytokines such as interleukin (IL) 1β, IL-6, and tumor necrosis factor α (TNF-α) (16,17). These cytokines impair neuronal survival by dysregulating BDNF (brain-derived neurotrophic factor) and CREB (cyclic-AMP response-element binding protein)—key regulators of neuronal plasticity and neurogenesis (13,18,19). In addition, preclinical studies suggest that neuroinflammation contributes to depressive-like symptoms by disrupting neurotrophic signaling (20–22).

LPS administration in rodents induces neurobehavioral deficits similar to those seen in neurodegenerative conditions, which are reversible with anti-inflammatory and antidepressant treatments (23,24). However, most LPS studies have used a single dose and assessed behavioral and molecular changes within 24 to 72 hours of administration, a period normally associated with significant LPS-induced sickness behavior (18,25–27). While LPS-induced sickness behavior—characterized by lethargy, reduced food intake, and fever—typically resolves after this period (26,27), it remains unclear whether reported neurobehavioral changes stem from acute systemic inflammation rather than chronic neuroinflammation. Limited studies have explored repeated LPS administration beyond 7 days (13,28), despite evidence that recurrent peripheral inflammation can induce long-term (LT) microglial

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activation, and hence neuroinflammation (29). Since neurodegenerative disorders arise from chronic, rather than transient neuroinflammation, evaluating the LPS model's validity requires distinguishing sickness behavior from sustained neuroinflammatory effects. In addition, the brain's compartmentalization plays a critical role in studying neuroinflammatory effects, as it contributes to the differential clinical presentations observed in neurological disorders. Therefore, identifying region-specific changes in molecular pathways susceptible to LPS-induced dysregulation is essential for understanding its contribution to the development of depressive-like symptoms.

In this study, we investigated changes in behavior and brain region–specific changes in neurotrophic, inflammatory, and apoptotic molecular markers following acute and repeated LPS administration after the resolution of initial sickness behavior. We showed that behavior was differentially affected, depending on the exposure time to systemic inflammation. Although neuroinflammation was present in both the acute and repeated LPS exposure studies, neurotrophic factors were dysregulated more substantially in chronic inflammation and in select brain regions associated with depressive-like symptoms.

METHODS AND MATERIALS

Ethical Approval

Ethical clearance was obtained from the Animal Ethics Screening Committee of the University of the Witwatersrand (2022/05/03C) following South African animal welfare regulations. In vivo experiments comply with Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

Animals

Thirty-seven 12-week-old male Sprague Dawley rats (250 ± 50 g) were sourced from the Wits Research Animal Facility. Rats were acclimatized for 7 days before testing. Experimental animals were housed individually under standard conditions (25 ± 1 °C, 24%–26% humidity) with ad libitum water and rat chow (Epol) on a 12-hour light/dark cycle (lights on at 7:00 AM; testing from 7:30 AM). Neurobehavioral tests were conducted in a separate testing room.

Study Design

Experimental animals were randomly divided into short-term (ST) (n = 18) and long-term (LT) (n = 19) groups and were further divided into saline control (CON) (n = 10 for ST and LT) or LPS-treated groups (n = 8 for ST, n = 9 for LT) (Figure 1A). Control rats received saline (0.1 mL, intraperitoneally [i.p.]), while LPS rats received 1 mg/kg LPS (*Escherichia coli* serotype 0111: B4; Sigma-Aldrich Chemical Corp.) in sterile saline (0.9% NaCl). ST-LPS rats received a single injection, while LT-LPS rats were injected once weekly for 4 weeks. Sickness behavior was monitored postinjection, with a 72-hour recovery period prior to neurobehavioral testing; tests were conducted once in ST groups and weekly in LT groups (Figure 1A, B).

Neurobehavioral Assessments

A modified sucrose preference test (SPT) was used to assess anhedonia-like behavior in control and LPS-treated rats (30,31). Additionally, the open field test (OFT) was used to assess locomotor activity and anxiety-like behavior (32) (see Supplemental Methods for a detailed description).

Brain Tissue Collection

ST rats were terminated 7 days after saline or LPS administration, and LT rats 7 days after their final injection. Rats were briefly exposed to isoflurane anesthesia and decapitated using a rodent guillotine. Trunk blood was collected and stored at −80 °C. The brain was dissected into Hyp, Pfc, Str, Hip, Mdb, Ctx, and Cbm and then snap frozen in liquid nitrogen and stored at −80 °C for molecular analysis.

Circulating Inflammatory Marker Concentrations

Systemic inflammation was confirmed by measuring serum IL-1β, TNF-α, and IL-6 protein concentrations in duplicate using enzyme-linked immunosorbent assays (Elabscience). Local inflammation was determined by hippocampal and striatal IL-1β protein concentrations (ABclonal). Detection ranges were 31.25 to 2000 pg/mL for IL-1β and 12.5 to 800 pg/mL for TNF-α and IL-6, respectively, with a <10% coefficient of variation. Reagents and serum samples were brought to room temperature for 20 minutes, and all reagents were prepared according to manufacturer instructions (see Supplemental Methods for additional information).

Gene Expression of Brain Tissue Molecular Markers

Real-time polymerase chain reaction (RT-PCR) was performed on brain tissues to assess *Bdnf*, *Creb*, *Nt-3*, *Ngf*, *Il6*, *Il1β*, *Tnf-α*, *Ifn-γ*, *Bax*, and *Bcl2* expression using RT-PCR TaqMan assays (Thermo Fisher Scientific), with *Tbp* as the endogenous control. Amplification was conducted on a StepOne RT-PCR System (Applied Biosystems), and messenger RNA (mRNA) expression changes were calculated using the $2^{-\Delta\Delta CT}$ method, expressed as fold change (FC) relative to control (33) (see Supplemental Methods for additional information).

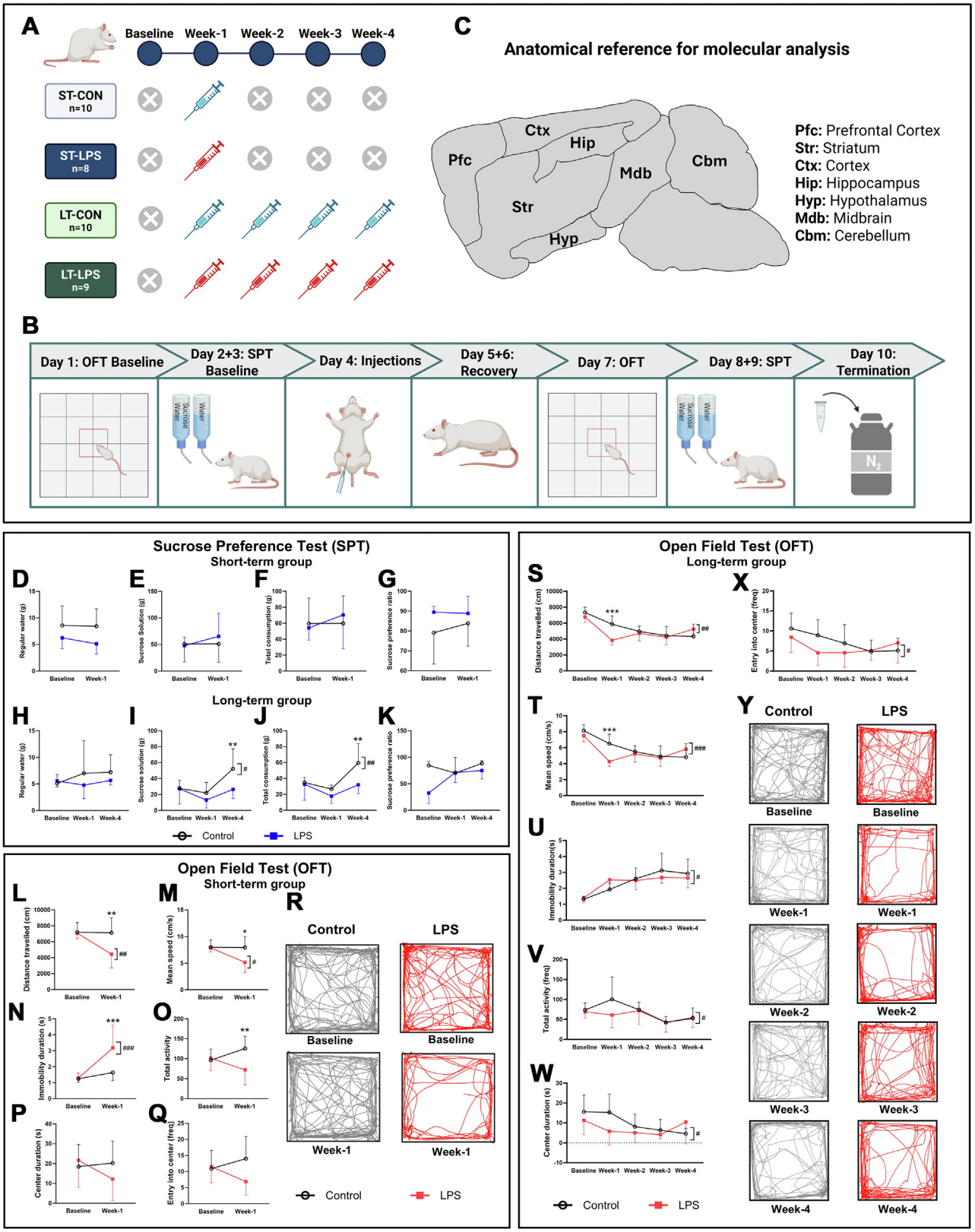
Detection of Vacuolation and Immune Infiltration in the Rat Brain

Sagittal sections were prepared from frozen brain tissue (5 μm) using a Leica CM1860 cryostat (Leica Biosystems), stained with hematoxylin and eosin, and examined for histopathological changes using a Leica DVM6 digital microscope.

Data Analysis

Data analysis was performed using SAS version 9.4 (SAS Institute Inc.), with results presented as mean ± SD. SPT and OFT differences were assessed using repeated-measures mixed-model analysis of variance (ANOVA, generalized linear model procedure), while molecular data were analyzed via 2-way ANOVA with treatment and time as main effects, followed by Tukey's post hoc test.

Heatmaps of gene expression z scores (see Supplemental Methods for additional information) for ST- and LT-CON and LPS-groups were generated in GraphPad Prism version 10 (GraphPad Software). Log_2 FCs for molecular and behavioral outcomes were computed as the ratio of ST- and LT-LPS to ST- and LT-CON averages and presented as a volcano plot. Pearson's correlation analysis was used to assess


A
Baseline
Week-1
Week-2
Week-3
Week-4
ST-CON n=10
ST-LPS n=8
LT-CON n=10
LT-LPS n=9
C
Anatomical reference for molecular analysis
Ctx
Hip
Pfc
Mdb
Cbm
Str
Hyp
Pfc: Prefrontal Cortex
Str: Striatum
Ctx: Cortex
Hip: Hippocampus
Hyp: Hypothalamus
Mdb: Midbrain
Cbm: Cerebellum
B
Day 1: OFT Baseline
Day 2+3: SPT Baseline
Day 4: Injections
Day 5+6: Recovery
Day 7: OFT
Day 8+9: SPT
Day 10: Termination
Sucrose
Water
N2
Sucrose Preference Test (SPT)
Short-term group
D
E
F
G
Regular water (g)
Sucrose Solution (g)
Total consumption (g)
Sucrose preference ratio
Baseline
Week-1
Long-term group
H
I
J
K
Week-4
Control
LPS
Open Field Test (OFT)
Short-term group
L
M
R
Distance travelled (cm)
Mean speed (cm/s)
Control
LPS
Baseline
Week-1
N
O
Immobility duration (s)
Total activity
P
Q
Center duration (s)
Entry into center (freq)
Open Field Test (OFT)
Long-term group
S
X
Distance travelled (cm)
Entry into center (freq)
Baseline
Week-1
Week-2
Week-3
Week-4
T
Y
Mean speed (cm/s)
Control
LPS
U
Immobility duration(s)
V
Total activity (freq)
W
Center duration (s)
Control
LPS

associations between molecular and behavioral outcomes. Statistical significance was set at $p < .05$.

RESULTS

Repeated LPS Administration Causes Anhedonia-Like Behavior in the LT but Not the ST Group

Rats were treated with either LPS (1 mg/kg, i.p.) or saline (0.1 mL, i.p.) once in the ST groups or once weekly for 4 weeks in the LT groups (Figure 1A). Neurobehavioral tests were performed 72 hours postinjection. No differences were found in regular water consumption (Figure 1D, H) or sucrose preference ratio (Figure 1G, K) in either the ST or the LT groups (all *p*s $> .05$). No differences were observed in sucrose solution volume or total consumption between ST-CON and ST-LPS groups (all $p > .05$). However, the LT-CON group consumed significantly more sucrose water (Figure 1I) at week 4 than the LT-LPS group ($p = .0097$) and increased total consumption (Figure 1J) from baseline ($p = .0052$), week 1 ($p < .0001$), and LPS week 4 ($p = .0019$). These results suggest that anhedonia-like behavior is more likely to occur with chronic, rather than ST, systemic inflammation.

In the OFT, the ST-LPS group had a significant decrease in total distance traveled (Figure 1L) compared with baseline ($p = .0058$) and the ST-CON group ($p = .0021$). Consequently, the mean speed (Figure 1M) of ST-LPS rats at week 1 was also lower than both baseline ($p = .0110$) and ST-CON rats ($p = .0054$). This decrease in locomotion resulted in a decrease in total activity (Figure 1O, R) of ST-LPS rats ($p = .006$) and a corresponding increase in immobility duration (Figure 1N) ($p = .0006$) compared with ST-CON rats. Despite these behavioral alterations, there were no significant differences in either the time spent in the center of the arena (Figure 1P) or the frequency of center entries (Figure 1Q, R) between the ST-LPS and ST-CON groups.

In the LT groups, the LPS rats initially showed a significant decrease in total distance traveled (Figure 1S) at week 1 ($p < .0001$), which persisted at week 2 ($p = .0004$), week 3 ($p < .0001$), and week 4 ($p = .0267$) compared with baseline. This progressive decline in movement was accompanied by a reduction in mean speed (Figure 1T), which was significantly lower than baseline at week 1 ($p < .0001$) and week 3 ($p < .0001$). When compared with LT-CON rats, both the total distance traveled (Figure 1S) and mean speed (Figure 1T) were significantly lower only at week 1 (both *p*s = .0004), indicating that the initial effect of LPS exposure was more pronounced. A significant increase in immobility duration (Figure 1U) was observed at all time points in both LT-LPS and LT-CON groups (all *p*s $< .05$), paralleled with a decrease in total activity (Figure 1V) over time (all *p*s $< .05$). In the LT-CON group, the time spent in the center of the arena (Figure 1W) and the frequency of center entries (Figure 1X) decreased over time ($p < .05$), but was not shown in the LT-LPS rats ($p > .05$). Interestingly, at week 1, LT-LPS rats displayed lower total activity (Figure 1V), reduced center duration (Figure 1W), and fewer center entries (Figure 1X) compared with LT-CON rats, although these differences were not statistically significant ($p = .1089$, $p = .2607$, and $p = .0953$, respectively).

LPS-Induced Systemic Inflammation Increases *Il6* and *Tnf-α* mRNA Expression in All Brain Regions

To confirm the presence of systemic inflammation, serum inflammatory marker concentrations were measured. LPS-administered animals had higher circulating IL-1β (Figure 2A) and TNF-α (Figure 2B) concentrations in the ST ($p = .0409$ and $p < .0001$, respectively) groups than their respective control groups. Rats in the ST-LPS group had higher circulating IL-1β (Figure 2A) and TNF-α (Figure 2B) concentrations than LT-LPS rats ($p = .0084$ and $p < .0001$, respectively). IL-6 concentration (Figure 2C) was higher in the LT-LPS ($p < .0001$) but not in the ST-LPS ($p = .9712$) group compared with the control groups. There were no significant differences in IL-1β levels in the Hip or Str of ST and LT rats (all *p*s $> .05$) (Figure S1A, B).

LPS-induced neuroinflammation was confirmed in several brain regions by measuring the expression of proinflammatory cytokines. The mRNA expression of *Il1β* (Figure 2D, G), *Tnf-α* (Figure 2E, H), and *Il6* (Figure 2F, I) were significantly increased in the ST-LPS rats compared with ST-CON rats in all brain regions. *Il1β* (Figure 2D) was higher in the LT-LPS rats than in the LT-CON rats in all brain regions (all *p*s $< .05$) except Str, where the expression was higher in LT-CON rats than in LT-LPS rats ($p = .0005$). *Tnf-α* mRNA expression (Figure 2E) was higher in the Hyp ($p = .0006$), Mdb ($p = .0001$), Ctx ($p = .0033$), and Cbm ($p = .0002$) in the LT-LPS group than in the LT-CON group. Interestingly, *Il6*

Figure 1. Study design and neurobehavioral analysis in ST- and LT-treated LPS rats compared with controls. **(A)** Treatment timeline and **(B)** study design: Pretreatment baseline values were recorded for the OFT and SPT. Rats in the ST group received a once-off injection on day 4, of either LPS (1 mg/kg, i.p.) or saline (CON; 0.1 mL, i.p.), completed the OFT (72 hours later) and SPT (on days 8 and 9), and were terminated on day 10. Rats in the LT group received either LPS or saline once a week for 4 weeks. LT rats were subjected to the OFT 72 hours after each of the 4 injections and completed the SPT after receiving their first injection (week 1) and then again after their final injection (week 4), after which they were terminated (end of week 4). **(C)** Histological reference showing the sagittal view of the rat brain with regions that were collected for molecular analysis. **(D–Y)** Effect of LPS treatment (1 mg/kg, i.p.; ST, $n = 8$; LT, $n = 9$) or saline (0.1 mL, i.p.; ST, $n = 10$; LT, $n = 10$) on SPT and OFT measurement time points include baseline (pre-LPS treatment measurement, $N = 37$); week 1: after 1 dose of LPS (ST, $n = 18$; LT, $n = 19$); week 2: second LPS dose (LT, $n = 19$); week 3: third LPS dose (LT, $n = 19$); week 4: fourth and final LPS dose (LT, $n = 19$). SPT showing the effect of LPS in the ST **(D–G)** and LT **(H–K)** groups on regular water consumption **(D, H)**, sucrose solution consumption **(E, I)**, total volume of water and sucrose solution consumed **(F, J)**, and the sucrose preference ratio **(G, K)**. OFT showing ST **(L–R)** and LT **(S–Y)** groups illustrating the effect of LPS on distance traveled **(L, S)**, mean speed **(M, T)**, duration immobile **(N, U)**, total activity **(O, V)**, time spent in the center of the arena **(P, W)**, and frequency of entry into the center **(Q, X).** Group representative track plots **(R, Y)** showing movement in the OFT of LPS and control rats at different time points in the ST and LT treatment groups. Data are presented as mean ± SD. Data were analyzed using a repeated-measures 2-way analysis of variance followed by a Tukey's post hoc test. In the SPT, $p < .01$ (**) control week 4 vs. LPS week 4, $p < .05$ (#) control week 4 vs. control and LPS over time, $p < .01$ (##) control week 4 vs. control and LPS over time. In the OFT, $p < .05$ (*) control vs. LPS, $p < .01$ (**) control vs. LPS, $p < .001$ (***) control vs. LPS, $p < .05$ (#) LPS vs. treatment over time, $p < .01$ (##) LPS vs. treatment over time. CON, control; i.p., intraperitoneally; LPS, lipopolysaccharide; LT, long-term; ST, short-term. [Figure was created with BioRender.com.]

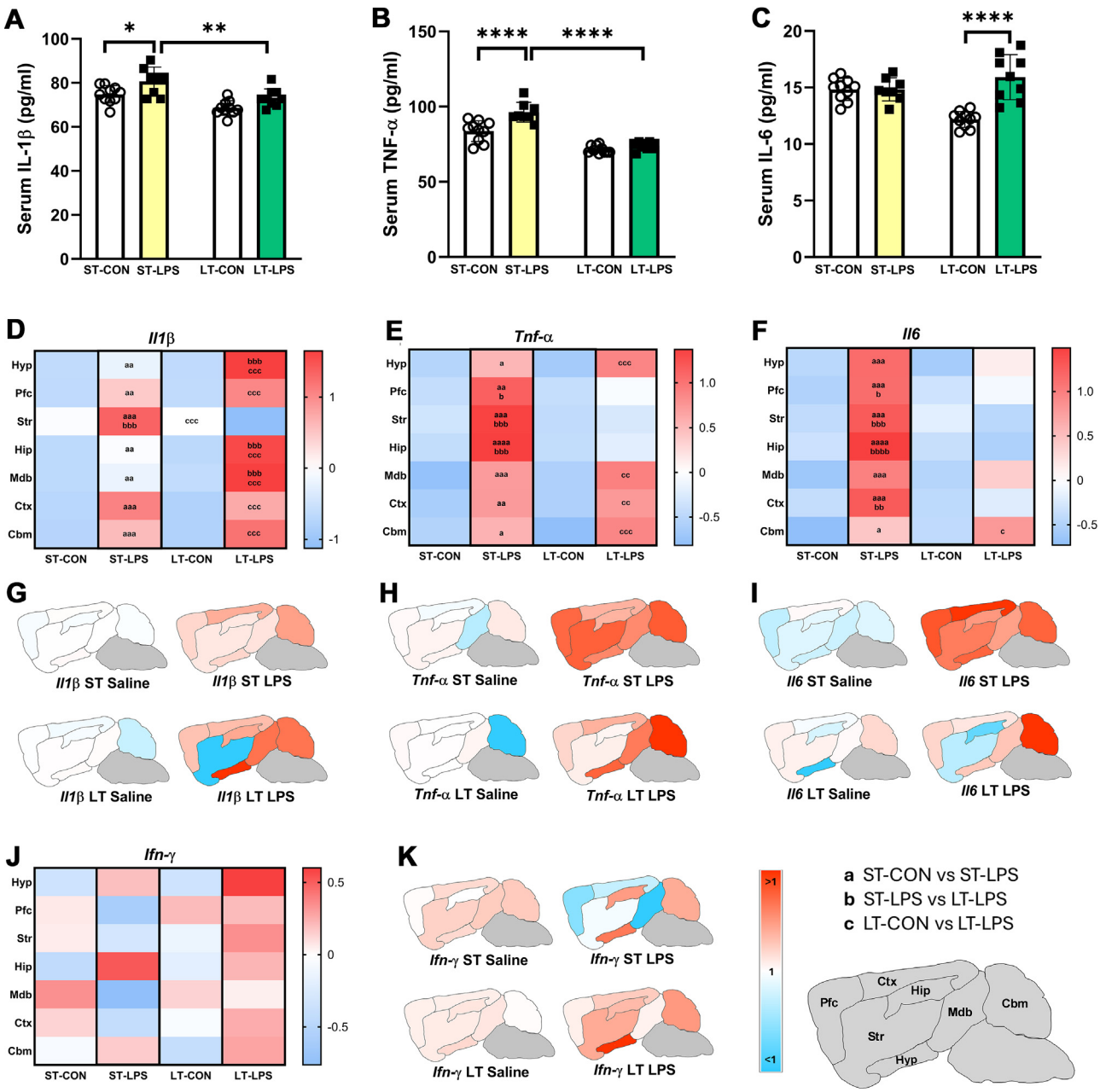


Figure 2. Circulating inflammatory cytokines and brain tissue expression of inflammatory and apoptotic markers. Serum concentrations of IL-1β **(A)**, TNF-α **(B)**, and IL-6 **(C)** in the ST- and LT-LPS and CON groups. **(D–K)** Heatmaps of real-time polymerase chain reaction results showing the effect of ST- and LT-LPS treatment on the mRNA expression of inflammatory markers in the Hyp, Pfc, Str, Hip, Mdb, Ctx, and Cbm. **(G–I, K)** Heatmaps of the sagittal plane of the rat brain showing upregulated or downregulated mRNA expression in the different brain regions as a result of LPS administration. LPS (1 mg/kg, i.p.; ST, $n = 8$; LT, $n = 9$) and saline (0.1 mL, i.p.; ST, $n = 10$; LT, $n = 10$) were administered once off (ST; $n = 18$) and once a week for 4 weeks (LT; $n = 19$). Data are presented as mean ± SD relative to the housekeeping gene *Tbp*. Data were analyzed using a 2-way analysis of variance followed by a Tukey's post hoc test, and heatmaps are represented as expression values (*z* scores) for differentially expressed genes. **(A–C)** $p < .05$ (*), $p < .01$ (**), $p < .0001$ (****). **(D–F, J) a** ST-CON vs. ST-LPS; **b** ST-LPS vs. LT-LPS; **c** LT-CON vs. LT-LPS. $p < .05$ (a, b, c), $p < .01$ (aa, bb, cc), $p < .001$ (aaa, bbb, ccc), $p < .0001$ (aaaa, bbbb, cccc). Cbm, cerebellum; CON, control; Ctx, cortex; Hip, hippocampus; Hyp, hypothalamus; IFN-γ, interferon gamma; IL, interleukin; i.p., intraperitoneally; LPS, lipopolysaccharide; LT, long-term; Mdb, midbrain; mRNA, messenger RNA; Pfc, prefrontal cortex; ST, short-term; Str, striatum; TNF-α, tumor necrosis factor α.

mRNA expression (Figure 2F) was not increased in the LT-LPS rats compared with the LT-CON rats in any brain regions (all *p*s > .05) except in the Cbm ($p = .0188$). Additionally, *Il1β* mRNA expression was higher in the Hyp, Hip, and Mdb (all *p*s < .0001) of the LT-LPS compared with the ST-LPS groups, whereas *Tnf-α* and *Il6* mRNA expression were higher in the ST-LPS than in the LT-LPS group in the Pfc, Str, and Hip (all *p*s < .05). There were no significant

differences in the mRNA expression of *Ifn-γ* (Figure 2J, K) in any of the brain regions of ST or LT animals.

The ratio of the *Bax/Bcl2* mRNA expression (Figure 3A, B), a marker of apoptosis, showed no differences in the ST-LPS versus ST-CON groups. There was a significant increase in the *Bax/Bcl2* ratio in the LT-LPS group compared with the LT-CON group in the Hyp (p = .0358) and the Str (p = .0404) and between the LT-LPS and ST-LPS groups in the Ctx (p = .0246). Hippocampal sections from saline CON rats showed normal neuronal morphology (Figure 3C). In contrast, LPS administration led to noticeable vacuolation (Figure 3D) and immune cell infiltration (Figure 3D). These findings suggest that LPS exposure was associated with significant immune activation and neurodegeneration.

There were no significant differences in *Bdnf* mRNA expression (Figure 4A, E) between the ST-CON and ST-LPS groups in any of the brain regions (all ps $>$ .05). However, *Bdnf* mRNA expression (Figure 4A) was significantly increased in the LT-LPS group compared with the LT-CON and ST-LPS groups in the Ctx (p = .0032 and p $<$ .0001, respectively) and Cbm (both ps = .004).

Creb mRNA expression (Figure 4B, F) was significantly higher in the ST-LPS rats compared with the ST-CON rats in the Hyp (p = .0106), Pfc (p = .0134), Str (p = .0045), Hip (p $<$ .0001), and Mdb (p = .0077) and also compared with the LT-LPS in the Pfc (p = .0089), Str (p = .0024), and Hip (p $<$ .0001). There were no significant differences in the mRNA expression of *Creb* between the LT-LPS and LT-CON groups (all ps $>$ .05).

The mRNA expression of *Nt-3* (Figure 4C, G) was significantly increased in LT-LPS rats compared with LT-CON rats in the Pfc (p = .0002), Hip (p = .0171), Ctx (p = .0002), and Cbm (p $<$ .0001) and compared with ST-LPS rats in the Pfc (p = .0001), Hip (p = .0254), and Cbm (p $<$ .0001). *Nt-3* mRNA expression was not

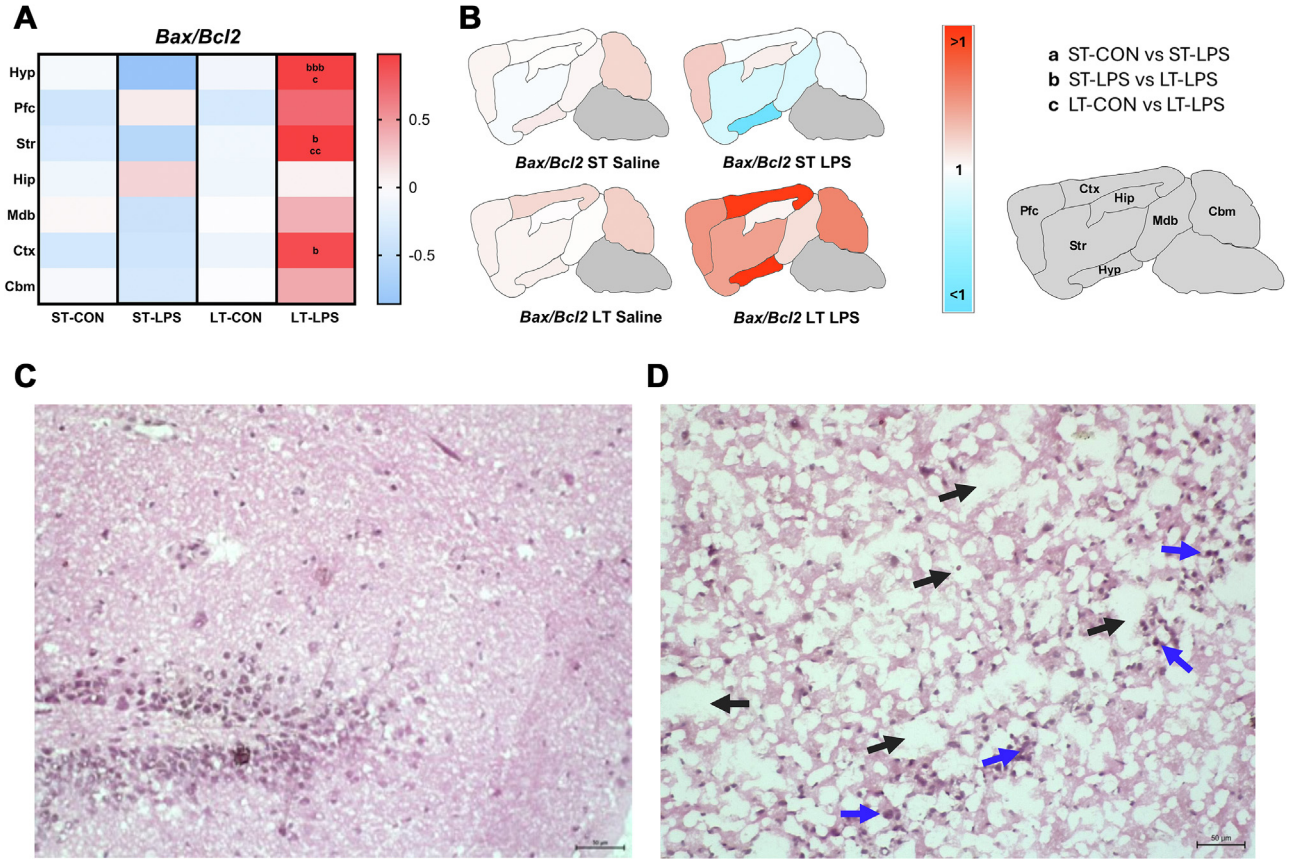


Figure 3. Brain tissue expression of apoptotic markers and H&E stained sections of rat sagittal hippocampus. **(A)** Heatmap of real-time polymerase chain reaction results showing the effect of ST- and LT-LPS treatment on the mRNA expression of apoptotic markers in the Hyp, Pfc, Str, Hip, Mdb, Ctx, and Cbm. **(B)** Heatmaps of the sagittal plane of the rat brain showing upregulated or downregulated mRNA expression in the different brain regions as a result of LPS. LPS (1 mg/kg, i.p.; ST, n = 8; LT, n = 9) and saline (0.1 mL, i.p.; ST, n = 10; LT, n = 10) were administered once off (ST; n = 18) and once a week for 4 weeks (LT; n = 19). Data are presented as mean ± SD relative to the housekeeping gene *Tbp*. Data were analyzed using a 2-way analysis of variance followed by a Tukey's post hoc test, and heatmaps are represented as expression values (z scores) for differentially expressed genes. **(A) a** ST-CON vs. ST-LPS; **b** ST-LPS vs. LT-LPS; **c** LT-CON vs. LT-LPS. p $<$ .05 (a, b, c), p $<$ .01 (aa, bb, cc), p $<$ .001 (aaa, bbb, ccc), p $<$ .0001 (aaaa, bbbb, cccc). **(C, D)** H&E staining of rat sagittal hippocampus (scale bars = 50 μm) after treatment with **(C)** saline (0.1 mL, i.p.) CON showing the normal structure, **(D)** LPS (1 mg/kg, i.p.) showing vacuolated cells (black arrows) and immune cell infiltration (blue arrows). Bax, Bcl-2-associated X protein; Bcl2, B-cell leukemia/lymphoma 2 protein; Cbm, cerebellum; CON, control; Ctx, cortex; H&E, hematoxylin and eosin; Hip, hippocampus; Hyp, hypothalamus; i.p., intraperitoneally; LPS, lipopolysaccharide; LT, long-term; Mdb, midbrain; mRNA, messenger RNA; Pfc, prefrontal cortex; ST, short-term; Str, striatum.

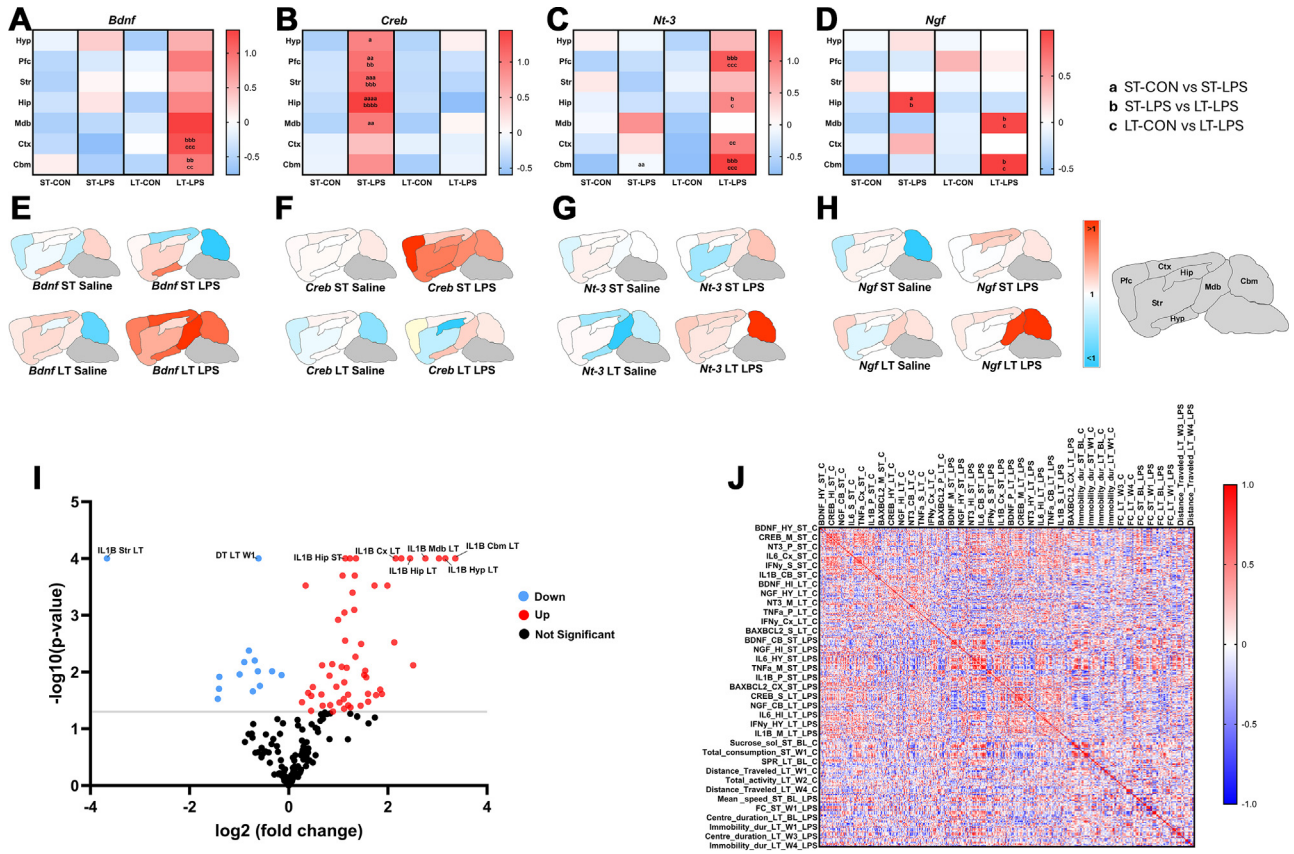


Figure 4. Brain tissue mRNA expression of neurotrophic factors and associations of molecular and behavioral outcomes in LPS treatment. Heatmaps of real-time polymerase chain reaction results showing the effect of ST- and LT-LPS treatment on the mRNA expression of *Creb* **(B, F)** and neurotrophic markers **(A, C, D, E, G, H)** in the Hyp, Pfc, Str, Hip, Mdb, Ctx, and Cbm. **(E–H)** Heatmaps of the sagittal plane of the rat brain showing the upregulated or downregulated mRNA expression in the different brain regions as a result of LPS. LPS (1 mg/kg, i.p.; ST, $n = 8$; LT, $n = 9$) and saline (0.1 mL, i.p.; ST, $n = 10$; LT, $n = 10$) were administered once off (ST; $n = 18$) and once a week for 4 weeks (LT; $n = 19$). Data are presented as mean ± SD relative to the housekeeping gene *Tbp*. Data were analyzed using a 2-way analysis of variance followed by a Tukey's post hoc test, and heatmaps are presented as expression values (*z* scores) for differentially expressed genes. **(A–D) a** ST-CON vs. ST-LPS; **b** ST-LPS vs. LT-LPS; **c** LT-CON vs. LT-LPS. $p < .05$ (a, b, c), $p < .01$ (aa, bb, cc), $p < .001$ (aaa, bbb, ccc), $p < .0001$ (aaaa, bbbb, cccc). **(I)** Volcano plot of all behavioral and molecular data with filtering criteria of $|\log_2 FC| > 1.3$ and $p < .05$, and **(J)** Pearson's correlation coefficient matrix heatmap of all molecular and behavioral analysis; $p < .05$. *Bdnf*, brain-derived neurotrophic factor; Cbm, cerebellum; CON, control; *Creb*, cyclic-AMP response-element binding protein; Ctx, cortex; FC, fold change; Hip, hippocampus; Hyp, hypothalamus; *Ifn-γ*, interferon gamma; *Il*, interleukin; i.p., intraperitoneally; LPS, lipopolysaccharide; LT, long-term; Mdb, midbrain; mRNA, messenger RNA; *Ngf*, nerve growth factor; Pfc, prefrontal cortex; ST, short-term; Str, striatum.

different between the ST-LPS and ST-CON groups, except for higher expression in the Cbm ($p = .0011$).

Ngf mRNA expression (Figure 4D, H) was significantly increased in the Hip of ST-LPS rats compared with ST-CON rats ($p = .036$) and LT-LPS ($p = .0383$) rats. Interestingly, *Ngf* mRNA expression was significantly increased in the Mdb and Cbm of LT-LPS rats compared with LT-CON rats ($p = .0462$ and $p = .0285$, respectively) and ST-LPS rats ($p = .0164$ and $p = .0261$, respectively). These results suggest that neurotrophic factor expression is differentially affected by acute and chronic inflammation in a region specific matter, with distinct patterns observed across different neurotrophic factors.

Multivariate analysis of all neurobehavioral and molecular data (Figure 4I) shows that 14 features were lower while 55 features were higher in the LT-LPS group. Of these features, the region-specific increase in expression of *Il1β* was the most significant contributor to the differences between the groups. Furthermore, *Il1β* mRNA expression in the various brain regions was significantly associated with changes in the expression of various molecular markers and in neurobehavioral outcomes including an increase in apoptotic marker expression (LT- and ST-LPS Hip, Mdb, Cbm) changes in *Bdnf* (LT-LPS Hip and Pfc), *Creb* (LT- and ST-LPS Hip, Pfc, and Cbm), *Nt-3* (ST-LPS Hyp, Hip, and Mdb), *Ngf* (LT- and ST-LPS Hip, and Str), *Tnf-α* (LT-LPS Hyp, Pfc, and ST-LPS Hip, Cbm), *Il6* (LT- and ST-LPS Hip, Mdb); sucrose solution and total consumption (LT-LPS Hip, Str, and ST-LPS Hyp, Mdb, Cbm); center duration (LT-LPS Hip, Mdb, Ctx, and ST-LPS Hyp, Cbm); distance traveled; and mean speed (LT-LPS Pfc) (Figure 4J).

DISCUSSION

Neuroinflammation contributes to the progression of neurodegenerative disorders (34). The LPS model has been instrumental in understanding the role of inflammation in the development of these conditions (35–37). However, few studies have evaluated the contribution of neuroinflammation to neurobehavioral and brain molecular changes independent of acute sickness behavior (18,27,38). Here, we used a chronic LPS model to investigate the effects of prolonged systemic inflammation on neurobehavior and brain region–specific molecular changes. We administered a single LPS dose and terminated animals after 7 days (ST-LPS) or gave LPS weekly for 4 weeks. Behavioral tests were performed 72 hours postinjection to ensure that sickness behavior had been resolved.

Phenotypic characterization showed that ST-LPS exposure did not affect anhedonia-like behavior, but LT-LPS rats exhibited reduced pleasure-seeking behavior at 4 weeks. In the SPT, a decreased preference for sucrose water over normal drinking water is considered a sign of anhedonia-like behavior (30). In the present study, while sucrose preference ratios remained unchanged, absolute sucrose consumption was lower in LT-LPS rats than controls. Interestingly, no differences were observed 1 week after a single LPS dose, consistent with studies that have shown no acute LPS effects on sucrose intake (39). In contrast, some studies have reported decreased sucrose preference 24 hours after single or repeated LPS doses (13,18,25,40,41), likely reflecting sickness behavior rather than depressive-like symptoms. Anhedonia presentation varies in neurological disorders, where some patients with major depressive disorder seek high-caloric foods while others lose interest (42). Clinical studies suggest that anti-inflammatory treatments can alleviate anhedonia in treatment-resistant depression, highlighting a bidirectional relationship between inflammation and pleasure-seeking behavior (43,44).

The OFT assessed locomotor activity as a measure of anxiety-like behavior, a symptom of neuronal dysfunction (45). A single LPS dose resulted in a decreased distance traveled, mean speed, and total activity while immobility increased after 7 days in both ST-LPS and LT-LPS groups. These results are consistent with some (38,46,47) but not other (17,18,26) previous studies. Acute inflammation causes symptoms such as lethargy and malaise, resulting in decreased locomotor activity (48,49), which could be misinterpreted as depressive-like behavior. Interestingly, with repeated LPS administration, there were no differences in locomotor activity between the control and LPS groups. However, both groups showed decreased movement over time, suggesting tolerance to LPS-induced effects, possibly due to adaptive immune responses (29,50) or habituation to the testing environment (51,52).

Overall, our findings suggest that acute LPS exposure decreases locomotor activity, but does not induce anhedonia, while chronic inflammation may alter pleasure-seeking behavior. Repeated LPS exposure may lead to immune adaptation, thereby preventing sustained anxiety-like behavior. Nevertheless, neuroinflammatory disorders are largely driven by molecular changes in the brain that may underpin phenotypic changes.

The present study confirmed LPS-induced systemic inflammation, evidenced by increased circulating IL-1β and TNF-α but not IL-6 concentrations in the ST-LPS group. However, IL-1β protein concentrations in the Hip and Str remained unchanged across groups. The lack of change in protein concentrations may be due to tight translational regulation, where increased mRNA levels do not always lead to higher protein expression. Factors such as microRNAs, RNA-binding proteins, ribosomal activity, and rapid protein degradation may prevent IL-1β accumulation to detectable levels (53,54). Interestingly, IL-6 concentrations were elevated only in the LT-LPS group. LPS-induced systemic inflammation is known to increase BBB permeability, allowing circulating cytokines to enter the brain and trigger a localized immune response (55,56). We observed increased *Tnf-α* expression across all brain regions in the ST-LPS group, with the highest levels in the Hip and Str, consistent with studies that have linked LPS-induced TNF-α upregulation to sickness behavior and cognitive impairments (57,58). This effect is initiated by the binding of LPS to TLR4 on the surface of microglia (17), which activates the MyD88 pathway, causing a rapid activation of NF-κB (58–60). With repeated LPS administration, *Tnf-α* expression was also increased in the Hyp, Mdb, Ctx, and Cbm. When elevated levels of *TNF-α* persist, especially in the Hyp and Mdb, it can lead to neurodegeneration, which accelerates gliosis and increases oxidative stress (61,62). LPS-activated microglia produce more *TNF-α* by adopting a proinflammatory (M1) phenotype, releasing reactive oxygen species (ROS) and nitric oxide, further damaging neurons (63–65). TNF-α also increases extracellular glutamate signaling by stimulating the TNFR1 in astrocytes to induce glutamate exocytosis and inhibit reuptake of glutamate (63). In the neuron, through its interaction with the TNFR1, TNF-α decreases the surface expression of inhibitory $GABA_A$ (gamma-aminobutyric acid A) receptors and increases Ca^{2+} permeable–AMPA receptors and NMDA receptors, resulting in excitatory potentiation and excitotoxicity (63). Elevated Ca^{2+} levels also generate ROS, impairing glutamate transport into neighboring astrocytes, resulting in cellular damage or neuronal death (63,66). As neurons undergo degeneration, microglia remain in an activated state, perpetuating the release of TNF-α (63). Chronic TNF-α elevation across brain regions has been linked to AD and PD (61,62,67), suggesting that prolonged LPS-induced neuroinflammation may contribute to neurodegenerative disease progression by disrupting the excitation-inhibition balance.

In this study, *Il1β* expression was increased in all brain regions of the ST-LPS and the LT-LPS groups. Interestingly, *Il1β* expression was decreased in the Str of LT-LPS rats compared with control and ST-LPS rats, while the Hyp, Hip, and Mdb showed a 2-fold increase in LT-LPS rats compared with ST-LPS rats. These findings highlight the region-specific and time-dependent role of IL-1β, which may have both protective and harmful effects (68–72). Similar to previous reports, the highest FC in *Il1β* expression was in the Hyp of the LT-LPS group (72,73). Indeed the Hyp is responsible for regulating several physiological processes, such as hormonal release from the pituitary gland, managing behaviors related to thirst, sleep, hunger, stress, and temperature regulation (72,73). Dysregulation of this pathway is linked to depressive symptoms, and elevated IL-1β has been associated with anhedonia,

disrupted sleep, and other depressive-like behaviors (72,74–76). Our results also suggest a link between reduced sucrose-seeking behavior in the SPT and *Il1β* expression. In addition, IL-1β may contribute to neurotoxicity by stimulating excessive glutamate production via mitochondrial glutaminase upregulation, leading to neuronal cell death (71,77). In the current study, *Il1β* expression was associated with changes in *Bax* (pro-apoptotic) and *Bcl2* (anti-apoptotic), with a higher *Bax/Bcl2* ratio indicating greater susceptibility to apoptosis and neurodegeneration (69,78). Chronic LPS administration increased this ratio in the Hyp, Str, and Ctx, suggesting that these regions are particularly susceptible to LPS-induced neurodegeneration. This pro-apoptotic state may be driven by elevated *Tnf-α* and *Il1β*, as these cytokines have been linked to oxidative stress and mitochondrial dysfunction, which are key triggers of apoptosis in LPS models (79). Additionally, vacuolation and immune cell infiltration in the Hip of LPS-treated rats indicate potential immune mediated neurodegenerative processes.

Interestingly, circulating IL-6 concentrations did not increase; however, *Il6* mRNA expression was increased in all brain regions of the ST-LPS rats. In contrast, in the LT-LPS rats, circulating IL-6 concentrations were increased, while *Il6* expression was not increased in the brain. These contrasting findings of IL-6 regulation in ST and LT inflammation may provide evidence for the dual role of IL-6 in neuronal degeneration and neuronal survival (80,81). It has been suggested that IL6 exerts both anti- and proinflammatory effects in the brain (82). Furthermore, IL6 plays a critical role in neurogenesis by acting as a neurotrophin (82). Increased *Il6* corresponded with elevated *Ngf, Bdnf*, and *Nt-3*, which support neuronal survival. IL-6 activates the Jak/Stat pathway, promoting the proregenerative effects of NGF, BDNF, and NT-3 (81,83). We showed increased *Bdnf* expression in the Pfc, Hip, Mdb, Ctx, and Cbm in the LT-LPS group, while *Nt-3* expression was increased in the Mdb and Cbm of the ST-LPS rats and in the Pfc, Hip, Ctx, and Cbm of the LT-LPS rats. Therefore, the unexpected increase in these neurotrophins may be driven by *Il6* signaling, suggesting a region-specific protective role.

Alternatively, the increased neurotrophic factor expression in the LT-LPS groups may be explained by development of tolerance to repeated LPS exposure (29,84), potentially driven by neurotrophic pathways that enhance neurotrophin secretion, such as BDNF, during neuroinflammation (85). BDNF and CREB signaling share a bidirectional relationship (86), with CREB regulating neurogenesis and gene expression in dopaminergic neurons (87). In this study, *Creb* expression increased across multiple brain regions in ST-LPS rats, particularly in the Hip, suggesting a compensatory response to inflammation-induced cell damage. Since CREB is rapidly phosphorylated during acute neuroinflammation, modulating synaptic plasticity and memory (88), its upregulation may help counteract neuronal damage. Overall, our findings suggest that *Tnf-α* and *Il1β* contribute to neurodegeneration through apoptosis, while *Il6* may offer neuroprotection via interactions with neurotrophins. This study provides molecular insights into LPS-induced neuroinflammation; however, future research should include a comprehensive battery of neurobehavioral tests to assess depressive-like behaviors and minimize learning effects in the OFT. Our findings may also be male specific, since females are known to show a stronger peripheral inflammatory response to LPS due to estrogen effects (89–92). In this study, we aimed to assess the effects of LPS independent of estrogen, however future studies should explore estrogen's role in neuroinflammation and behavior. Additionally, we were unable to differentiate between pro- and mature BDNF, and future studies would benefit from additional molecular analyses to distinguish between the two. The current study showed that the Str was differentially affected by LPS compared with other brain regions, warranting further investigation into monoamine signaling and the expression of excitotoxic receptors. Lastly, future research should explore the role of neurotrophins in immune tolerance during chronic LPS exposure, offering insights into their neuroprotective effects in neuroinflammation.

Conclusions

This study provides valuable insights into LPS-induced neuroinflammation, which may improve the clinical translation of this model. Our results suggest that, independent of sickness behavior, acute and chronic systemic inflammation may be differentially associated with various depressive-like behaviors and region-specific changes in the expression of inflammatory, apoptotic, and neurotrophic molecular markers. This suggests that systemic inflammation may contribute to neurodegeneration through increased activation of the *Il1β* pathway, while *Il6* may have a protective neurotrophic role.

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LP, AMEM, and SB contributed to writing the original draft of the article, reviewing and editing the article, data curation, and formal analysis. LP, AR, AMEM, and SB contributed to methodology and investigation. AMEM and SB contributed to data visualization. AMEM and SB contributed to supervision, resources, project administration, funding acquisition, and conceptualization. AMEM and SB contributed to software.

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