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Behavioral and monoamine perturbations in adult male mice with chronic inflammation induced by repeated peripheral lipopolysaccharide administration

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Abstract

Considering the limited information on the ability of chronic peripheral inflammation to induce behavioral alterations, including on their persistence after inflammatory stimuli termination and on associated neurochemical perturbations, this study assessed the effects of chronic (0.25 mg/kg; i.p.; twice weekly) lipopolysaccharide (LPS) treatment on selected behavioral, neurochemical and molecular measures at different time points in adult male C57BL/6 mice. Behaviorally, LPS-treated mice were hypoactive after 6 weeks, whereas significant hyperactivity was observed after 12 weeks of LPS and 11 weeks after 13 week LPS treatment termination. Similar biphasic responses, i.e., early decrease followed by a delayed increase were observed in the open field test center time, suggestive of, respectively, increased and decreased anxiety. In a forced swim test, mice exhibited increased immobility (depressive behavior) at all times they were tested. Chronic LPS also produced persistent increase in splenic serotonin (5-HT) and time-dependent, brain region-specific alterations in striatal and prefrontocortical dopamine and 5-HT homeostasis. Microglia, but not astrocytes, were activated by LPS early and late, but their activation did not persist after LPS treatment termination. Above findings demonstrate that chronic peripheral inflammation initially causes hypoactivity and increased anxiety, followed by persistent hyperactivity and decreased anxiety. Notably, chronic LPS-induced depressive behavior appears early, persists long after LPS termination, and is associated with increased splenic 5-HT. Collectively, our data highlight the need for a greater focus on the peripheral/central monoamine alterations and lasting behavioral deficits induced by chronic peripheral inflammation as there are many pathological conditions where inflammation of a chronic nature is a hallmark feature.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Keywords

Lipopolysaccharide; Inflammation; Depressive-like behavior; Splenic serotonin

1. Introduction

Contrary to earlier views on the central nervous system (CNS) as an immunologically privileged site, accumulating evidence highlights the existence of an extensive dynamic bidirectional interaction between the immune system and the CNS [1–4]. From a neuropathological perspective, peripheral inflammation can aggravate an ongoing neurological damage and exaggerate motor and/or cognitive impairments in patients with neurodegenerative diseases, such as Parkinson's disease (PD) and Alzheimer's disease (AD) [5–7]. In animal models, peripherally-generated inflammatory mediators induced by systemic challenge with lipopolysaccharide (LPS) or double-stranded RNA (poly I:C), representative of, respectively, bacterial and viral infections, have been found to increase brain inflammatory cytokines, resulting in progressive neurotoxicity [8, 9].

Peripheral LPS induced alterations in central monoamine homeostasis have been reported [10, 11]. Specifically, single peripheral LPS administration increases the turnover of dopamine (DA), norepinephrine (NE) and serotonin (5-HT) in several different brain regions [12, 13]. Behaviorally, acute peripheral LPS administration causes sickness behavior in rodents [14, 15] that is characterized with decreased exploratory activity, locomotion and social exploration [16–18]. Of note, if the immune system activation persists, sickness behavior can transition into a depressive-like behavior [19, 20]. There is a partial symptoms-overlap between inflammation-induced sickness and depressive-like behaviors, but their course is quite different. Sickness behavior is an acute, quick, transient response to infections/inflammas and it is reversible; depressive behavior is characterized by a delayed onset and a long-lasting nature, which results when activation of the innate immune response is exaggerated in intensity and/or duration and it is observed in the absence of concurrent increased circulating inflammatory cytokines [19, 21, 22].

Epidemiological investigations of the relationship between chronic inflammation and depression indicate increased rate of depressive disorders in patients suffering from chronic inflammatory conditions, as well as in patients undergoing interferon alpha (IFN α) therapy [23–25]. Few animal studies have reported behavioral deficits, including depressive-like behavior and impaired spatial memory following chronic central (intra-CNS) or peripheral LPS administration [26–29], but the laboratory studies that have investigated the impact of chronic peripheral inflammation on the induction of depression-like behavior are limited. Moreover, only limited information [30, 31] exists on other motor and non-motor behavioral domains, including anxiety, and on associated neurochemical changes following long-term peripheral LPS administration. Also, data regarding the time-dependent changes in behavior, i.e., whether inflammagen (LPS)-induced initial behavioral impairments are persistent throughout the time course of chronic inflammatory stimulation or after treatment termination, are limited.

Besides inducing depression and anxiety, peripheral inflammatory events can influence the etiology and progression of many ongoing degenerative diseases, including AD and PD [7, 32]. Chronic central LPS challenge models have successfully replicated key components of the characteristic neurodegenerative pattern seen in PD and have demonstrated significant early microglial activation followed by delayed and time-dependent nigral dopaminergic neuron degeneration [33, 34] and a long-term disruption of AD-relevant hippocampal network activity [35, 36]. Peripheral administration of a single high dose (5 mg/kg) of LPS resulted in long-lasting neuroinflammation and nigral dopaminergic neurodegeneration [37]. Data that chronic peripheral LPS administration can cause both neuroinflammation and progressive dopaminergic neuron loss similar to PD are very limited [31, 38] and indicate that a genetic susceptibility might be necessary for the full pathology to occur when the inflammatory stimulus is at a lower level and of chronic nature [31].

Besides causing neuroinflammation, acute peripheral LPS administration also induces inflammatory cytokines expression in peripheral tissues, such as spleen and liver. Interestingly, chronic mild stress induced dysregulation of the anti/inflammatory cytokine balance in different brain regions and spleen was implicated in the induction of depressive-like behavior in a rat study [39]. Spleen, the secondary lymphoid organ, is largely innervated by noradrenergic fibers of the sympathetic nervous system and functions as a platelet storage site [40]. The neural signals transmitted by NE (major neurotransmitter of the sympathetic nervous system) are received by platelets where this neural input is converted to immunomodulatory signals by platelet-released 5-HT [40]. Importantly, acute central administration of immune mediators, such as interleukin-1 beta (IL-1 β) or prostaglandins, causes activation of sympathetic nerves, resulting in altered splenic tissue levels of NE and/or 5-HT [41, 42]. However, limited information exist on splenic monoamine alterations (if any) induced by chronic peripheral inflammation and their potential contribution in the induction of depressive-like behavior.

Considering all of the aforementioned data gaps and the high prevalence of chronic inflammatory conditions of bacterial origin [43, 44], the main objectives of the current study were to investigate the effect of chronic peripheral LPS treatment in adult male C57BL/6 mice on selected behavioral, neurochemical, and molecular parameters. To assess the potential neurobehavioral consequences of chronic LPS, we employed a battery of behavioral tests that could effectively assess both the locomotor (open field, grip strength and pole tests) as well as depressive-like (forced swim test) alterations. Additionally, to gain an insight into the persistent effects of repeated peripheral LPS administration, the behavioral, neurochemical and molecular analyses were performed on separate set of mice chronically treated with LPS, followed by a substantial wait period during which treatment was discontinued.

2. Materials and methods

2.1. Reagents

Unless otherwise stated, all chemicals including lipopolysaccharide *Escherichia coli*, serotype 0111:B4 (LPS) were purchased from Sigma (St. Louis, MO).

2.2. Animals

Animals were male C57BL/6 mice (4–5 months old; Taconic, Hudson, NY) weighing 30.10 ± 0.25 g (mean $\pm$ SEM) prior to treatment and were housed ($n = 5$ /cage) with food and water available *ad libitum* on a 12-h light/dark cycle in an AAALAC accredited facility throughout the study. All procedures involving animal handling were in accordance with the latest NIH guidelines and were approved in advance by the Institutional Animal Care and Use Committee (IACUC) of the University of Georgia.

2.3. Animal treatment

Mice ($n = 15$ per group) were injected intraperitoneally (i.p.) with sterile normal saline (vehicle) or LPS at a dose of 0.25 mg/kg body weight (BW) twice weekly for up to 25 weeks. LPS/saline administration occurred at midmorning on the same two days (consistently spaced apart) of each week throughout the entire treatment duration. The dose and regimen of LPS treatment for this study was based on recently published chronic peripheral inflammatory models that have shown neuroinflammation behavioral alterations, and nigrostriatal dysfunction (genotype-dependent) in mice [31, 38, 45]. Behavioral tests (described in detail below) were carried out (24 h after the last injection) at 6 and 12 weeks ($n = 5$ per group) post LPS/saline treatment on the same sets of mice. Randomly selected subsets ($n = 5$ per group) from each of the saline or LPS groups were sacrificed (24 h after the last injection) after 13 or 25 weeks of the treatment. Additionally, another subset of mice ($n = 5$ per group) were given LPS/vehicle for 13 weeks followed by a 12 week period without any treatment; these mice were sacrificed after the 12 week wait period (13 week on + 12 week off). Behavioral tests of these mice, which were 6- and 12-week behaviorally-experienced, were done 1 week prior to the sacrifice, i.e., 13 week on + 11 week off. A detailed outline of the experimental design is presented in Fig. 1A. BW was recorded once weekly for the entire experimental period (25 weeks). Food intake and water intake were measured weekly up to the 12th week of study duration. Organs (brain, liver, spleen, and thymus) were harvested, weighed and quickly frozen at -80°C for further analyses. Molecular tests, including ELISA and qPCR, were conducted after 13 or 25 weeks of LPS treatment, as well as 12 weeks after termination of the 13-week LPS treatment.

In order to assess the acute effect of LPS administration on peripheral inflammatory response, an additional experiment was performed where mice ($n = 4$ /group/time point) were given a single i.p. injection of sterile normal saline (vehicle) or LPS (0.25 mg/kg BW) followed by sacrifice at 2, 5 and 24 h post LPS administration; plasma was collected from these mice and analyzed for tumor necrosis factor alpha (TNF α) and interleukin 6 (IL-6) using ELISA.

2.4. Behavior

The behavioral tests consisted of open field, pole test, grip strength, and forced swim tests; all mice were subjected to tests in the same order. These tests were performed in succession over 2 days (open field, pole test, grip strength on day 1 and forced swim test on day 2) in a designated behavioral testing room located nearby, but separate, from that in which animals were housed. All animals were initially naive to the behavioral apparatuses and the experimenter who conducted the behavioral tests was treatment-blinded. This behavioral

testing paradigm used frequently and is similar to our earlier studies, i.e. [46, 47]. Generally, if the same group of animals is used for multiple complex behavioral tasks, a one week inter-test interval has been used as a rule of thumb to minimize the carry over effects, but this interval is recommended in cases where the behavioral test batteries include relatively more stressful, tasks such as passive avoidance and/or Morris water maze tests [48]. The tests we used are less intensive than the frequently used Functional Observational Battery (FOB) testing procedure that is often paired with an open field testing on the same day [49]. Importantly, mice subjected to six behavioral tests with a 1–2 day inter-test interval showed similar behavioral performance compared to those which underwent the same number of behavioral tests carried out with an inter-test interval of 1 week, prompting the authors to advocate the use of a rapid test battery to make the behavioral phenotyping of mice more efficient and less time consuming [50].

2.4.1. Open field—Each mouse was individually monitored in an open field arena (l × w × h: 25 × 25 × 40 cm, divided into 16 square grids; Coulbourn Instruments, Whitehall, PA) for 30 min with Limelight video tracking software (Actimetrics, Wilmette, IL). Total distance traveled (cm) was recorded as a measure of locomotor activity and analyzed per 5 min intervals. Additionally, the times spent in the periphery or in the center of the open field arena were measured as one indicator of anxiety and analyzed per 5 min intervals [51].

2.4.2. Pole test—Pole test was conducted to assess mice's motor coordination as detailed in [46]. After a 5-min resting period (following the open field test), mice were placed upright on the top of a gauze-wrapped vertical metal pole (1 cm in diameter and 55 cm in height). The maximum turning time allowed was 60 s and the total time (complete turn + descent) per trial was 120 s [52]. If a mouse did not turn within the first 60 s, it was gently guided and a maximum measurement (60 s) was recorded. A total of 4 trials were completed for each mouse at each time point with a 3–5 min inter-trial interval and the average turn, descent time, and total times spent on the pole from all 4 trials were used for statistical analysis [53].

2.4.3. Grip strength—This test (10 min rest after the pole test) was performed to assess neuromuscular function by measuring the forelimb grip strength using mouse-specific strength gauge (Bioseb, France) as previously described [46]. Average maximum grip force (recorded in newtons [N]) of four trials was used for statistical analysis.

2.4.4. Forced swim test (FST)—FST was performed as described previously [46]. Mice were placed gently in a large cylindrical container (18 cm in diameter and 25 cm in height) filled approximately two-thirds with tap water (3 L, 29±1 °C) for a period of 15 min. Limelight video tracking software (Actimetrics) was utilized to score the total times spent swimming, climbing, or immobile and analyzed per 5 min intervals.

2.5. HPLC-ECD

Monoamine analysis by HPLC-ECD was performed as described in detail in [54]. Briefly, brain (micropunches from prefrontal cortex [PFC], striatum, and hippocampus) and spleen (20 mg) tissue samples were placed in 100 and 200 µl of 0.2 N perchloric acid, respectively,

sonicated and centrifuged. A 20 µl aliquot of the supernatant was injected into HPLC with an electrochemical detector (ECD) (Waters Alliance, Waters Co., Milford, MA) to determine: (1) DA and its metabolites dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT); (2) NE and its metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG); and (3) serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in brain tissues. 5-HT and NE levels were determined in splenic tissue. Prior to statistical analysis, brain neurotransmitter/metabolite data were normalized on a per mg protein basis [47]; splenic 5-HT and NE data were normalized on a per mg tissue basis.

2.6. ELISA

Plasma concentrations of tumor necrosis factor- α (TNF α) and interleukin-6 (IL-6) were analyzed after acute (2, 5 and 24 h post LPS injection) and chronic LPS (13 week, 25 week and 13 week on +12 week off) administration using mouse-specific ELISA kits (R&D Systems, Minneapolis, MN) as we have described it before [55]. Samples and standards (TNF α : 2000-31.25 pg/ml) and (IL-6: 1000-15.625 pg/ml) were run in duplicate. Absorbance (450 nm analytical read; 570 nm background correction read) was measured using a Synergy 4 hybrid multi-mode microplate reader (BioTek Instruments, Winooski, VT) and the mean from each sample replicate was used for statistical analysis. Samples with cytokine levels below the lower limit of detection (LOD; LODs for IL-6 and TNF α were 2.5 and 5.2 pg/ml, respectively) were assigned the LODs for statistical purposes.

2.7. Real-time quantitative PCR (qPCR)

qPCR was done as we have described it before [47]. Briefly, total RNA from substantia nigra samples was isolated using E.Z.N.A. microelute total RNA kit (Omega Bio-Tek, Inc., Norcross, GA) and quantified using Take 3 plate and Epoch microplate spectrophotometer (Biotek, Winooski, VT). Using a Peltier thermal cycler (Bio-Rad; 5 min 25 °C, 30 min 42 °C, and 5 min 85 °C), 75 ng RNA was converted to cDNA with qScript cDNA SuperMix (Quanta Bioscience, Gaithersburg, MD). Using 0.5 ng of cDNA per sample, expression of tyrosine hydroxylase (TH), dopamine receptor-2 (D2DR), glial fibrillary acidic protein (GFAP; astrocyte activation marker), F4/80 (microglia activation marker), TNF α , IL-6 and interleukin-1 β (IL-1 β) were determined by qPCR using mouse-specific, certified primers (Supplemental Table 1) and SYBR Green (Qiagen, Valencia, CA). Amplifications were performed in a Mx3005P qPCR machine (Stratagene) programmed for an initial warming (10 min, 95 °C) followed by 45 cycles (15 s, 95 °C, 1 min, 60 °C) with each sample run in triplicate. Treatment differences were calculated as a fold change using the C_t method with β -actin as the house-keeping gene [46].

2.8. Statistical analysis

Two-way analysis of variance (ANOVA) was conducted to analyze the BW (treatment $\times$ duration [week] with treatment as the between-subject factor and duration as the within-subject factor), weekly food (g/kg BW) and water (ml/kg BW) (treatment $\times$ duration [week] with treatment as the between-subject factor and duration as the within-subject factor) intake and the open field (treatment $\times$ time [interval] with treatment as the between-subject factor

and time as the within-subject factor) data. One-way ANOVA or T-tests (as appropriate) were used to analyze the effect of LPS on organ weights (g/kg BW), behavioral endpoints (except open field), HPLC and molecular data within a time point. If ANOVA's overall main effect was significant ($p \leq 0.05$), treatment means were separated by Student-Newman-Keuls *post hoc* test. All results are presented as mean $\pm$ SEM and are considered significant at $p \leq 0.05$.

3. Results

3.1. Body weight (BW), food and water intakes, organ weights

Two-way ANOVA demonstrated an overall significant main effect of LPS ($F(1, 112) = 41.29, p \leq 0.001$) on BW up to the 13th week that was driven mainly by the effects of LPS during the first 4 weeks of the experiment where the BW decrease within each week was significant ($p \leq 0.05$); the largest BW drop (10%) occurred 1 week after LPS treatment initiation (Fig. 1B). Afterwards, BW of LPS-treated mice was only numerically ($p > 0.08$) smaller than the BW of saline controls except after the 8th week of LPS ($p \leq 0.05$). With respect to the BW from 13 to 25 weeks, while the saline and LPS-treated mice did not differ ($p > 0.25$; Fig. 1B), the BW of the mice in saline-13 week on+12 week off group increased significantly ($p \leq 0.05$) even 1 week after the discontinuation of saline injections and remained significantly elevated for the duration of the study (Fig. 1B). On the other hand, discontinuation of the LPS treatment (LPS-13 week on +12 week off group) did not affect the BW of the mice at any time point from week 14 to week 25 ($p > 0.20$; Fig. 1B). There was no significant effect of LPS ($F(1, 62) = 0.99, p > 0.30$) or treatment duration (week; $F(11, 62) = 0.59, p > 0.80$) with respect to the water intake, which was monitored during the first 12 weeks of the study (Fig. 1C). Similarly, no significant main effect of LPS ($F(1, 62) = 0.94, p < 0.35$ or treatment duration (week; $F(11, 62) = 0.63, p = 0.80$) was observed with respect to the food intake during the first 12 weeks of the study (Fig. 1C).

Thirteen week LPS administration produced slight, but significant ($p \leq 0.05$), increase in spleen weight; liver and thymic weights were unaffected ($p > 0.25$; Fig. 1D). Continued LPS treatment for another 12 weeks, i.e. a total of 25 weeks, moderately increased the liver weight ($p \leq 0.05$); weights of the other organs were not changed ($p > 0.25$; Fig. 1D). Compared to the 25-week saline group, LPS administration for 13 weeks followed by a 12 week wait period significantly increased the liver, spleen and thymic weights ($p \leq 0.05$; Fig. 1D).

3.2. Behavior

3.2.1. Open field—There were significant main effects of LPS ($F(1, 84) = 7.08, p \leq 0.01$) and interval (5 min time period; $F(5, 84) = 9.46, p \leq 0.001$), but without any significant interaction between the two ($F(5, 84) = 0.30, p > 0.90$) with respect to the distance traveled in the open field arena after 6 weeks of LPS treatment; LPS-treated mice were hypoactive as illustrated by the decreased distance traveled per 5 min interval ($p \leq 0.05$; Fig. 2A). As expected, both control and LPS-treated mice habituated to the arena over time and their overall activity decreased (e.g., distance traveled: 4054.77 ± 359.94 vs. 3637.27 ± 225.74 cm during the first 5 min and 2491.80 ± 210.94 vs. 1977.19 ± 224.36 cm during last 5 min

for control and LPS groups, respectively), with LPS-treated mice being hypoactive throughout the 30 min open field session. Six weeks later, i.e. after 12 weeks of LPS treatment, mice exhibited a significant increase in locomotor activity evidenced by the significantly increased distance traveled in the open field arena ($p = 0.01$; Fig. 2A). Compared to 6 weeks, as expected, the overall activity of both control and LPS groups was decreased (Fig. 2A). Control mice habituated quickly to the open field arena evidenced by 59% reduction in the locomotor activity as compared to the 6 week time point (Fig. 2A). However, in comparison to the 6 week treatment, 12 week LPS-treated mice had impaired long-term habituation evidenced by only 40% reduction in their locomotor activity (Fig. 2A). Meanwhile, within the 30 min open field session at 12 weeks, both control and LPS-treated mice habituated to the arena over time and their overall activity decreased (e.g., distance traveled: 1541.38 ± 532.50 vs. 2337.97 ± 57.00 cm during first 5 min and 856.99 ± 232.10 vs. 1727.78 ± 483.90 cm during last 5 min for control and LPS groups, respectively). A similar trend was observed even 11 weeks after the termination of 13 week chronic LPS treatment with LPS-treated mice exhibiting increased locomotor activity compared to the control group ($p = 0.05$; Fig. 2A).

There was also overall significant main effect of LPS with respect to the time spent in the center versus periphery of the open field arena after 6 weeks of LPS. The mean time spent (per 5 min interval) in the center was decreased ($p = 0.05$; Fig. 2B) and in the periphery was increased ($p = 0.05$; Fig. 2C). After 12 weeks of LPS treatment, mice did not show any significant difference with respect to the time spent in center (Fig. 2B) versus periphery (Fig. 2C) of the open field arena compared to the control group. However, the mean times spent in the center and periphery were increased (center; Fig. 2B) and decreased (periphery; Fig. 2C) significantly ($p = 0.001$) 11 weeks after the termination of the 13 week chronic LPS administration. Thirteen week LPS administration followed by an 11 week wait period also resulted in significant decrease in the corner time ($p = 0.05$; data not shown).

3.2.2. Pole test—No significant effect of LPS was observed with respect to the mean time to turn ($p > 0.90$), time to descend ($p > 0.70$) and total time ($p > 0.70$) after 6 weeks of LPS treatment (data not shown). The pole test parameters mentioned above were unaffected after 12 weeks of LPS administration and also 11 weeks after termination of a 13 week LPS treatment ($p > 0.25$; data not shown).

3.2.3. Grip strength test—Short of an apparent trend towards an increase in the maximum grip strength after 6 weeks on LPS ($p = 0.09$), the grip strength was unaffected after 12 weeks of LPS ($p > 0.20$) or 11 weeks after the discontinuation of a 13 week LPS treatment ($p > 0.90$; data not shown).

3.2.4. Forced swim test—After 6 weeks on LPS, mice swam significantly less ($p = 0.05$; Fig. 3A) and had a concomitant increase in the immobility time ($p = 0.05$; data not shown); the mean time spent climbing was unaffected ($p > 0.80$; Fig. 3B). Similarly, after 12 weeks on LPS, mice swam significantly less than control mice ($p = 0.05$; Fig. 3A) and had a corresponding increase in the immobility time ($p = 0.05$; data not shown); climbing was unaffected at this time point as well ($p > 0.10$; Fig. 3B). Eleven weeks after termination of the 13 week LPS treatment, mice continued to spend significantly less time swimming (p

0.05; Fig. 3A) with a parallel increase in the immobility time ($p = 0.05$; data not shown); at this time point, they also climbed less ($p = 0.05$; Fig. 3B).

3.3. HPLC

3.3.1. Spleen—Thirteen weeks of LPS treatment significantly reduced splenic NE ($p = 0.05$; Fig. 4A); however, NE levels remained unaffected after 25 weeks or 12 weeks after the termination of the 13 week LPS treatment ($p > 0.6$; Fig. 4A). With respect to 5-HT, LPS treatment produced a significant increase in splenic 5-HT levels after 13 or 25 weeks of LPS treatment; the increase was also present 12 weeks after discontinuation of the 13 week LPS treatment ($p = 0.05$; Fig. 4B).

3.3.2. Brain—In the PFC, 13 weeks of LPS treatment did not alter the levels of DA, 5-HT or their metabolites ($p > 0.40$; Table 1), but it did cause non-significant trend towards a decrease in the MHPG level ($p < 0.15$; Table 1) and in the MHPG/NE ratio ($p < 0.10$; data not shown), without affecting the parent neurotransmitter NE ($p > 0.50$; Table 1). 25 weeks of LPS administration did not alter the levels of DA, NE or their metabolites ($p > 0.45$; Table 1), but it significantly affected PFC 5-HT homeostasis. Specifically, LPS increased 5-HT and 5-HIAA levels ($p = 0.01$; Table 1), without affecting PFC 5-HIAA/5-HT ratio ($p > 0.25$; data not shown). Thirteen weeks of LPS treatment followed by a 12 week wait period caused moderate changes in PFC DA homeostasis; LPS caused an apparent trend towards a decrease in PFC DA ($p = 0.09$; Table 1), along with a numerical increase in the concentration of the DA metabolite, DOPAC ($p < 0.20$; Table 1). As a result, the PFC DOPAC/DA ratio was increased significantly ($p = 0.05$; data not shown). LPS did not increase the concentration of the other DA metabolite, HVA ($p > 0.50$; Table 1), but the HVA/DA ratio was increased to a level that was close to significance ($p = 0.07$; data not shown). LPS treatment for 13 weeks followed by a 12 week wait period also resulted in a numerical increase in PFC MHPG ($p = 0.13$; Table 1), as well as in an apparent trend towards an increase of the parent neurotransmitter NE ($p = 0.07$; Table 1); the MHPG/NE ratio was unaffected ($p > 0.70$; data not shown). No significant alteration of PFC 5-HT homeostasis was observed at this time point ($p > 0.50$; Table 1).

In the striatum, 13 week LPS treatment did not cause any alterations of DA or its metabolites ($p > 0.25$; Table 2); there was a numerical increase in the HVA/DA ratio ($p < 0.15$; data not shown). In contrast to DA, striatal 5-HT homeostasis was significantly affected after 13 weeks of LPS administration. Specifically, LPS increased striatal 5-HT ($p = 0.05$; Table 2) and caused apparent trends towards 5-HIAA increase ($p = 0.08$; Table 2) and a decrease in the 5-HIAA/5-HT ratio ($p = 0.10$; data not shown). Chronic LPS administration for 25 weeks significantly increased striatal concentration of the DA metabolite HVA ($p = 0.05$; Table 2), without affecting the level of DA itself ($p > 0.90$; Table 2). No significant alterations were observed with respect to striatal DOPAC ($p > 0.35$; Table 2) or the DOPAC/DA ratio ($p > 0.35$; data not shown). Striatal 5-HT homeostasis was not affected at this time point ($p > 0.35$; Table 2). LPS treatment for 13 weeks followed by a 12 week wait period did not affect striatal DOPAC ($p > 0.20$; Table 2) or DA ($p > 0.50$; Table 2), but the DOPAC/DA ratio was decreased ($p = 0.05$; data not shown). No significant changes were observed with respect to striatal HVA ($p > 0.60$; Table 2) or the HVA/DA

ratio ($p > 0.35$; data not shown). The concentration of the DA metabolite, 3-MT, which was only detected in the striatum, was unaffected after 13 or 25 weeks of LPS administration or 12 weeks after discontinuation of the 13 week LPS treatment ($p > 0.35$; data not shown). No significant alterations in striatal 5-HT or 5-HIAA levels were observed after LPS treatment termination ($p > 0.20$; Table 2).

Thirteen week LPS administration did not affect hippocampal monoamine homeostasis ($p > 0.25$; Table 3), except for numerical decrease in DA level ($p < 0.15$; Table 3). Similarly, LPS treatment for 25 weeks did not affect hippocampal levels of DA, NE, 5-HT or their metabolites ($p > 0.15$; Table 3). Similar to 13 week and 25 week data, hippocampal monoamine homeostasis remained unaffected 12 weeks after LPS treatment termination ($p > 0.15$; Table 3).

3.4. ELISA

Plasma IL-6 was undetectable in acute saline-treated mice at all time points (Fig. 5A). Single LPS administration increased plasma IL-6 after 2 or 5 h of LPS administration ($p < 0.05$; Fig. 5A); however, plasma IL-6 level was undetectable after 24 h (Fig. 5A). Similar to IL-6, plasma TNF α in saline-treated mice was not detectable at the three time points tested (Fig. 5B). Plasma TNF α was significantly elevated at 2 and 5 h ($p < 0.05$; Fig. 5B) post LPS injection and, similar to IL-6, was undetectable after 24 h (Fig. 5B).

After 13 weeks, plasma TNF α level in the LPS-treated mice was minimal and was comparable to that of saline-treated group ($p > 0.30$; Fig. 5C). Similar to the 13-week data, plasma TNF α was detectable only in few samples from 25 weeks or 13 weeks on + 12 weeks off LPS and it was not different from respective controls ($p > 0.30$; Fig. 5C). Plasma levels of IL-6 were below the detection limit at all three time points (data not shown).

3.5. qPCR

At the mRNA level in the substantia nigra, D2DR expression was significantly upregulated after 25 weeks of LPS administration ($p < 0.05$; Fig. 6); however, its expression was unaffected after 13 weeks of LPS treatment or 12 weeks after the termination of 13 week LPS administration ($p > 0.30$; Fig. 6). TH mRNA expression was unaffected irrespective of the treatment duration ($p > 0.30$; data not shown). LPS treatment for 13 or 25 weeks did not alter the GFAP mRNA expression ($p > 0.25$; Fig. 6); however, there was an apparent trend towards an increase in its expression 12 weeks after the 13 week LPS treatment termination ($p = 0.08$; Fig. 6). F4/80 mRNA level was upregulated ($p < 0.01$; Fig. 6) after 13 or 25 weeks of the LPS treatment, while no alteration was observed after the discontinuation of LPS treatment ($p > 0.40$; Fig. 6). The mRNA expression of the pro-inflammatory cytokines, TNF α , IL-6 or IL-1 β was unaffected by LPS ($p > 0.20$; data not shown), except for a moderate, but significant ($p < 0.05$) decrease in the IL-6 level observed 12 weeks after the termination of the 13 week LPS treatment (data not shown).

4. Discussion

Chronic peripheral inflammation has long been implicated in the etiology of immune-mediated diseases like rheumatoid arthritis and systemic lupus erythematosus [56, 57].

Besides peripheral immune dysregulation, inflammation-related events in the periphery can communicate with the CNS, producing an exacerbation of the CNS immune response and subsequent alterations in cognition, mood, and behavior [58]. Clinical evidence for a clear association between chronic, low-grade inflammatory response and depression, which is the most common mood disorder, is accumulating [59], but previous animal studies have largely focused on the impact of acute peripheral inflammation on emotional alterations. However, inflammation-associated emotional disorders, including depression in humans, are typically lifelong progressive conditions that are more likely to be related to a chronic inflammatory response [19]. We conducted this study to expand the limited experimental data on the impact of chronic peripheral inflammation on the induction of depressive behavior and other neurobehavioral alterations and on their persistence after termination of inflammatory stimuli exposure. The current study is unique in that it assessed the time-dependent changes in LPS-affected behavior and associated neurochemical and molecular substrates not only during the LPS treatment, but also after treatment termination in a chronic inflammatory model paradigm. The main findings of this study include: 1) Repeated peripheral LPS administration for 6 weeks resulted in depression-like emotional alterations, that persisted after 12 weeks of treatment and even long after termination of LPS administration; 2) chronic LPS caused brain region-specific and time-dependent monoamine alterations; 3) chronic LPS treatment increased splenic 5-HT, an effect that persisted long after LPS termination.

Previous rodent studies have shown that sickness behavior peaks 2–6 h after a single LPS challenge, is accompanied by increased peripheral inflammatory cytokines, and gradually fades over time, diminishing 24–48 h after LPS [16, 19, 60]. Consistent with these earlier findings, we found that plasma TNF α and IL-6 levels were significantly increased after 2 and 5 h, but not 24 h after a single LPS challenge. In light of this result, for the chronic experimental model adopted in this study, mice were given a 24 h rest period after the previous LPS administration and thereafter underwent behavioral tests or sacrifice in order to minimize the possible acute effects of LPS.

Acute LPS-induced sickness behavior can transform to a depressive-like behavior over time, which can persist even after the immediate behavioral response (reduction in locomotor activity) to LPS administration has normalized [16, 21, 22]. For example, 24 h after LPS treatment, while showing normal locomotor activity, mice exhibited increased immobility time in tail-suspension and forced-swim tests, indicative of depressive-like behavior [21]. It has to be noted that the above studies have adopted an acute inflammatory model paradigm to dissociate the LPS-induced sickness and depressive-like behaviors; the association between depression and chronic inflammation is still understudied, but evidence for it is mounting. Thus, the rates of depressive disorders in patients suffering from chronic inflammatory conditions as well as in patients undergoing IFN α therapy are higher [61–63].

In this study, we demonstrate that chronic LPS treatment results in depression-like emotional alterations (evidenced by the significantly decreased swimming time and concomitant increase in the immobility time in the forced swim test) which were apparent early on (6 weeks) and still present after 12 weeks of LPS treatment. Most intriguingly, we found that the depressive-like behavior persisted even long (11 weeks) after termination of

the chronic LPS administration, indicating the ability of prolonged LPS treatment to have a long-lasting, perhaps permanent, depressogenic effect. Interestingly, the long-lasting depressogenic effect observed in our study is in line with several case studies in humans where depressive symptoms are present even after termination of IFN α therapy, which causes enhanced production of inflammatory cytokines and dysregulates 5-HT homeostasis during the treatment period [62, 64–66].

Recent study reported chronic depression in female, but not male C57BL/6 mice, characterized by long-lasting anhedonic response (decreased sucrose preference) following repeated, intermittent LPS injection for 4 months [45]. Besides the different LPS treatment protocol used in [45], it is worth noting that studies in rats and mice have reported a clear sex-specific difference in sucrose consumption, with females showing greater sucrose preference than males [67, 68]. More importantly, male, but not female rats, showed complete absence of sucrose preference after an extended access to sucrose [68]. In this regard, it could be that in Kubera's study [45], the females were more responsive to the sucrose preference test or the long-term sucrose might have resulted in diminished sucrose preference in control male mice, which in turn have masked the effect of chronic LPS treatment in males.

In order to investigate potential peripheral monoamine alterations associated with the observed persistent depressive behavior, we assessed splenic NE and 5-HT levels; we found significant reduction in splenic NE after 13 weeks, but not after 25 weeks of LPS treatment or after LPS treatment termination. Interestingly, we observed a significant increase in splenic weight after 13 week of LPS treatment. Change in the tissue volume of lymphoid organs has been reported to affect the availability of NE as this neurotransmitter released from sympathetic nerves may have to travel larger distances to reach target cells in the lymphoid organs; hence, with the increase in splenic size, distant target cells are less likely to receive NE [69]. In this regard, given the lack of NE alterations after 25 week and long after the termination of the LPS treatment, it is likely that the decreased splenic NE observed after 13 week is merely a consequence of the increased splenic size, rather than an altered metabolism of NE. In contrast to NE, we observed consistent and lasting increase in splenic 5-HT. Although platelets cannot synthesize 5-HT, they can sequester large amount of 5-HT by an active uptake system mediated by NE [40]. Importantly, systemic inflammation induced by viral infections has been reported to cause platelet activation and enhanced splenic sequestration resulting in low levels of circulating platelets [70]. In this regard, the chronic LPS-induced elevation in splenic 5-HT level observed in this study is suggestive of an inflammation-dependent redistribution of platelets from blood to spleen and subsequent reduction in circulating platelets and platelet-associated 5-HT. Several studies have associated low levels of circulating platelet 5-HT with depression [71, 72]; however reports on the potential contribution of splenic serotonin in the induction of depressive behavior in an inflammatory setting is non-existent. Our study points to a close association between splenic serotonin 5-HT levels and depression, suggesting that the chronic peripheral inflammation-induced long-lasting depressive-like behavior could be associated with the LPS-induced net reduction in whole blood 5-HT and a simultaneous increase in splenic 5-HT level.

Besides splenic 5-HT dyshomeostasis, chronic LPS treatment produced time-dependent, brain region-specific impairment in 5-HT homeostasis. Specifically, the LPS-induced significant elevation in striatal (13 week) and cortical (25 week) 5-HT is indicative of an increased tissue 5-HT reserve so as to compensate for the augmented 5-HT demand (increased 5-HIAA levels) observed after 13 and 25 weeks of LPS treatment. Hence, the depressive behavior exhibited by mice at these time points could be a consequence of the LPS-induced augmentation of the 5-HT tissue reserve and subsequent reductions in the synaptic 5-HT levels. However, it is worth noting that although mice showed depressive-like behavior long after the discontinuation of LPS administration, brain 5-HT homeostasis was not altered at this stage. Therefore, it appears that although chronic LPS treatment impairs both central and peripheral 5-HT homeostasis, the peripheral (splenic) 5-HT alterations more closely align with the persistent depressive behavior observed in this study.

Increased anxiety has been reported to be highly comorbid with depression in patients suffering from chronic inflammatory conditions, as well as in patients undergoing IFN α -based immunotherapy [61, 73]. In our study, mice exhibited increased anxiety coupled with depressive-like behavior after 6 weeks of chronic LPS administration. Importantly, prolonged LPS administration for another 6 weeks, i.e. a total of 12 weeks only had a significant impact on depressive behavior and not on anxiety, indicative of a time-dependent dissociation of the behavioral pathologies in the continuum of neurological dysfunctions induced by chronic peripheral inflammation. Interestingly, mice exhibited a decreased anxiety level long after the termination of chronic low-dose LPS treatment. Taken together, our findings demonstrate a biphasic effect of chronic LPS administration on anxiety response with the initial increased anxiety-like behavior dissipating over time and further following an opposite trend (decreased anxiety) in the absence of an active peripheral inflammatory stimulus. Regardless, additional behavioral tests that are anxiety-centered, i.e., elevated plus maze or marble burying tests [51], may be employed in the future to further characterize this behavioral effect of chronic LPS treatment.

Reduction in locomotor activity following single LPS administration is a key behavioral response demonstrated in multiple studies [17, 18]. Here, we found that repeated LPS administration for 6 weeks reduced locomotion. However, with continued LPS treatment for another 6 weeks, i.e. a total of 12 weeks, mice displayed a significant increase in locomotion (compared to time-matched saline-treated controls) that persisted even long after the termination of LPS treatment. While the first 5 min of open field testing is typically used to assess novel environment exploration [74], an extended session length (usually for 30 min), is often required to assess the habituation of animals to the open field environment [51]. We found that within the 30-min session, both saline and LPS-treated mice habituated to the arena over time at all behavioral time points (6 weeks, 12 weeks and 13-week on +11-week off) tested. We also assessed the intersession habituation to the open field at the three behavioral testing times points by testing the same mice. Generally, with repeated open field testing rodents, especially C57BL/6 mice, will display intersession habituation, characterized by a general decrease in activity over time across sessions [75]. Interestingly, we found that compared to saline group, LPS-treated mice showed a 2-fold lesser decrease in long-term habituation at 12 and 24 week (13 week on +11 week off group). Increased familiarity of the open field test environment over repeated test sessions may reflect a form

of recognition memory [76]. Significant reduction in recognition memory following acute peripheral LPS administration has been demonstrated in several animal studies [77–79]. In this regard, the decreased long-term habituation over repeated open field testing exhibited by LPS-treated mice in this study could be due to the LPS-induced deficits in recognition memory; this needs to be verified by further testing.

It is noteworthy that the hyperactivity observed during chronic (12 week) LPS treatment occurred in the absence of an overt change in DA homeostasis in the multiple brain regions (PFC, striatum and hippocampus) assessed in this study. Hence, it is likely that the neurochemical changes in dopaminergic pathways, if at all present, might be too low to be detected in the regions we assessed, especially when measuring the tissue level of neurotransmitter/metabolites and not in vivo DA release by sensitive methods, such as microdialysis [80]. Alternatively, it is conceivable that unlike the acute effects, chronic LPS-induced neurochemical alterations involving dopaminergic pathways might be present in a highly region-dependent fashion and might have occurred in brain regions other than the ones we focused on. Interestingly, continued LPS administration for another 12 week, i.e. a total of 25 week, impaired DA homeostasis in the striatum indicative of a time-dependent region-specific neurochemical changes induced by chronic LPS treatment. Thirteen week of LPS treatment followed by 12 week wait period decreased DA level in PFC, which is indicative of increased DA utilization or increased synaptic DA release; the increased locomotor activity could be partly attributed to altered PFC DA homeostasis.

In addition to determining neurochemical alterations, in order to further elucidate the mechanism of LPS-induced locomotor impairments, we assessed the expression of the dopaminergic neuronal markers TH and D2DR in the substantia nigra. Nigral mRNA expression of TH and D2DR was not affected after 13 weeks and 12 weeks after the termination of 13 week chronic LPS treatment. However, after 25 weeks of continuous LPS administration, the nigral mRNA expression of D2DR was significantly upregulated, which is indicative of an impaired DA synthesis and release [81]. Overall, our findings suggest that the effects of chronic LPS on DA homeostasis are likely time- and brain-region-dependent.

To further investigate the possibility that the LPS-induced behavioral and neurochemical deficits might have resulted from an increased neuroinflammatory response, we analyzed the nigral mRNA expression of the microglia and astrocyte activation markers, F4/80 and GFAP, respectively, and of key pro-inflammatory cytokines. Interestingly, we found that chronic LPS administration produced microglial activation, evidenced by the significant upregulation of nigral F4/80 at 13 and 25 weeks post LPS treatment. However, in contrast to the LPS-induced microglial activation, nigral GFAP mRNA expression remained unaltered, indicating that the chronic LPS administration at this level mainly activates microglia. It is also worth mentioning that the significant enhancement in microglial activation observed at above two time points (13 and 25 weeks) in this study was not accompanied by an increase in the nigral expression of pro-inflammatory cytokines; other inflammatory mediators, such as prostaglandins or nitric oxide, could have been altered by LPS, but were not assessed.

Anhedonia, a core symptom of depression [82], has been demonstrated in cancer patients undergoing cytokine (IL-2 or IFN α) treatment [83]. Interestingly, systemic administration of

IL-2, but not IL-6 or IL-1 β induced anhedonic response, evidenced by decreased responding for rewarding hypothalamic self-stimulation in rats [84]. Additionally, abnormalities in the normal PFC or hippocampal functioning have been demonstrated in patients with mood disturbances, including major depressive disorders [85–87]. In this regard, the possibility of other microglial-derived cytokines, such as IL-2 and IFN α , and brain regions (other than what we assessed), mediating the LPS-induced depressive-like behavior observed in this study, cannot be discounted.

Higher levels of peripheral pro-inflammatory cytokine levels have been associated with depression [88, 89]. However, compared to the saline-injected group, we did not find any significant difference with respect to plasma TNF α and IL-6 levels, suggesting that the persistent depressive behavior exhibited by the mice in this study is not associated with increased circulating inflammatory cytokines. Previous studies have shown that repeated LPS administration can reduce the activation of immuno-inflammatory signaling pathways and can induce a phenomenon called endotoxin tolerance wherein animals become less responsive to subsequent LPS challenge [90–92]. Repeated peripheral LPS administration has been reported to produce time-dependent development of some behavioral tolerance, wherein the locomotor deficits observed at early time points disappear at a later time point of treatment [93, 94]. In our study, chronic LPS treatment did not induce behavioral tolerance as the LPS-treated mice showed locomotor and depressive-like deficits at multiple times during the treatment period as well as weeks after LPS treatment termination. Similar to our results, another group reported long-lasting depression in mice subjected to chronic peripheral LPS treatment [45]. Additionally, it is noteworthy that the behavioral deficits exhibited by the mice in our Kubera's [45] studies occurred in the absence of apparent elevated peripheral inflammatory response. These data are suggestive of development of peripheral, but not behavioral LPS tolerance. Importantly, studies have shown that in endotoxin-tolerant animals, although the peripheral effects of LPS on inflammatory processes are reduced/no longer present, higher levels of pro-inflammatory cytokines can still be present in the brain for a longer period resulting in long-lasting neurobehavioral deficits [45, 91]. Because we only assessed a set of inflammatory mediators in the periphery, it is also possible that other peripheral inflammatory mediators (other than what we assessed), such as IL-1 β or IL-12, might be contributing to the depressive behavior, as previously reported [95, 96]; this needs to be explored further.

5. Conclusions

In summary, we have shown that chronic peripheral LPS treatment induces a persistent state of depression in male C57BL/6 mice, lasting long after termination of the LPS treatment. Prolonged LPS treatment also affects monoamine pathways in a brain-region specific manner. The long-lasting depressogenic effect of chronic LPS are associated with persistently increased 5-HT in the spleen, which could lead to a subsequent decrease in circulating 5-HT levels. Collectively, the findings from this study suggest that repeated peripheral LPS administration can be used successfully to model chronic inflammation-induced depression and also emphasize the need to pay more attention on the peripheral/central monoamine alterations and lasting behavioral deficits induced by chronic peripheral

inflammation as there are many pathological conditions where chronic inflammation is a key feature.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Experimental design, body and organ weights, food and water intake. (A) Detailed outline of the study's experimental design. Adult male C57BL/6 mice ($n = 15/\text{group}$) were injected intraperitoneally (i.p.) with sterile normal saline (vehicle) or LPS at a dose of 0.25 mg/kg body weight twice weekly for up to 25 weeks. The same set of saline/LPS treated mice ($n = 5$ per group) was repeatedly tested at 6 weeks (behavior 1), 12 weeks (behavior 2) and 11 weeks after the termination of 13 week saline/LPS treatment (behavior 3). Randomly selected subsets ($n = 5/\text{group}$) of mice were sacrificed after 13 or 25 weeks of saline/LPS treatment and also 12 weeks after the termination of 13 week saline/LPS treatment (13 week on + 12 week off). Effect of saline or LPS treatment on (B) BW (g) of mice during the 25-weeks of treatment ($n = 5$ per group), (C) food (g/kg BW/week) and water intake (ml/kg BW/week) ($n = 15$ per group) during the 1st 12 weeks of treatment and (D) on organ (liver, spleen and thymus) weights (g/kg BW) in 13-week, 25-week and 13 week on +12 week off groups. Graphical representations are mean $\pm$ SEM. * $p < 0.05$ when compared to the saline group within a time point.

Fig. 2. Open field locomotor activity. Effect of LPS (0.25 mg/kg BW; i.p., twice weekly) treatment of adult male C57BL/6 mice after 6, 12 or 13-week on +11-week off LPS treatment on (A) distance traveled, (B) time spent in the center or (C) the periphery of the open field arena. Graphical representations are mean $\pm$ SEM ($n = 5$ /group). * indicates significant effect of LPS within a time point ($p < 0.05$). "a" indicates significant differences across different durations within a group ($p < 0.05$).

Fig. 3. Forced swim test. Effect of LPS (0.25 mg/kg BW; i.p., twice weekly) treatment of adult male C57BL/6 mice after 6, 12 or 13-week on +11-week off LPS treatment on (A) time spent swimming or (B) climbing. Graphical representations are mean $\pm$ SEM ($n = 5$ /group). * $p < 0.05$ when compared to the saline group within a time point.

Fig. 4. Splenic NE and 5-HT levels. Effect of LPS (0.25 mg/kg BW; i.p., twice weekly) treatment of adult male C57BL/6 mice on spleen (A) NE and (B) 5-HT level in 13-week, 25-week and 13-week on +12-week off groups. Graphical representations are mean $\pm$ SEM ($n = 5$ /group). * indicates significant effect of LPS within a time point ($p < 0.05$).

Fig. 5.

Plasma cytokine levels. Effect of acute LPS (0.25 mg/kg BW; i.p., twice weekly) administration to adult male C57BL/6 mice on plasma (A) IL-6 and (B) TNF α levels 2, 5 and 24 h post LPS injection as well as chronic LPS (0.25 mg/kg BW; i.p. twice weekly) administration on plasma (C) TNF α level in 13-week, 25-week and 13-week on +12-week off groups. Graphical representations are mean $\pm$ SEM ($n = 4-5$ /group). * indicates significant effect of LPS within a time point ($p < 0.05$).

Fig. 6.

Nigral mRNA expression. Effect of LPS (0.25 mg/kg BW; i.p.; twice weekly) treatment to adult male C57BL/6 mice after 13, 25 or 13-week on + 12 week off LPS treatment on mRNA expression levels of dopamine receptor-2 (D2DR), glial fibrillary acidic protein (GFAP) and F4/80 in the substantia nigra of adult male C57BL/6 mice ($n = 5$ per group). mRNA data are β -actin-normalized and are presented as fold change relative to respective control at each time point (mean $\pm$ SEM). * indicates significant effect of LPS ($p < 0.05$). ^ indicates significant trend ($p < 0.08$).

Table 1

Concentrations of monoamines and their metabolites in the prefrontal cortex (PFC) of male C57BL/6 mice treated with vehicle (saline) or LPS (0.25 mg/kg BW; i.p., twice weekly; $n = 5$ per group) in 13, 25 and 13-week on + 12-week off groups. Data are presented as mean $\pm$ SEM; unit: ng/mg protein.

Brain neurotransmitters and neurotransmitter metabolites								
Brain region	PFC							
Treatment	13-week							
		DA	DOPAC	HVA	NE	MHPG	5-HT	5-HIAA
Saline		0.56 ± 0.23	0.20 ± 0.12	0.47 ± 0.08	6.52 ± 0.28	6.73 ± 0.28	1.04 ± 0.06	0.53 ± 0.07
LPS		0.51 ± 0.15	0.19 ± 0.06	0.39 ± 0.09	6.71 ± 0.16	5.90 ± 0.38	0.97 ± 0.08	0.48 ± 0.05
25-week								
Saline		1.06 ± 0.21	1.70 ± 0.30	0.23 ± 0.02	9.32 ± 0.40	15.76 ± 2.17	1.01 ± 0.13	0.49 ± 0.06
LPS		0.97 ± 0.19	1.45 ± 0.09	0.25 ± 0.03	8.94 ± 0.39	14.74 ± 0.62	2.37 ± 0.34*	0.87 ± 0.07*
13-week on + 12-week off								
Saline		1.24 ± 0.45	1.69 ± 0.06	0.47 ± 0.12	8.34 ± 0.25	14.77 ± 0.89	1.07 ± 0.11	0.47 ± 0.04
LPS		0.34 ± 0.09	2.17 ± 0.29	0.59 ± 0.13	10.07 ± 0.81	16.76 ± 0.79	1.19 ± 0.15	0.47 ± 0.07

Abbreviations: PFC: prefrontal cortex; DA: dopamine; DOPAC: dihydroxyphenylacetic acid; HVA: homovanillic acid; NE: norepinephrine; MHPG: 3-methoxy-4-hydroxyphenylglycol; 5-HT: serotonin; 5-HIAA: 5-hydroxyindoleacetic acid.

* $p < 0.05$ compared to saline control.

Table 2

Concentrations of monoamines and their metabolites in the striatum of male C57BL/6 mice treated with vehicle (saline) or LPS (0.25 mg/kg BW; i.p., twice weekly; $n = 5$ per group) in 13, 25 and 13-week on + 12-week off groups. Data are presented as mean $\pm$ SEM; unit: ng/mg protein.

Brain neurotransmitters and neurotransmitter metabolites								
Brain region	Striatum							
Treatment	13-week							
		DA	DOPAC	HVA	NE	MHPG	5-HT	5-HIAA
Saline		252.72 ± 10.73	17.10 ± 0.75	17.19 ± 1.09	ND	ND	1.56 ± 0.38	2.10 ± 0.09
LPS		241.07 ± 11.16	17.17 ± 2.14	18.60 ± 0.76	ND	ND	4.43 ± 1.05*	2.51 ± 0.18
25-week								
Saline		201.82 ± 9.03	19.61 ± 0.90	12.64 ± 0.52	ND	ND	1.83 ± 0.22	2.02 ± 0.40
LPS		201.62 ± 10.68	18.46 ± 0.82	15.71 ± 0.95*	ND	ND	1.49 ± 0.20	1.56 ± 0.15
13-week on + 12-week off								
Saline		199.25 ± 6.72	20.58 ± 1.18	11.30 ± 0.96	ND	ND	1.91 ± 0.15	2.08 ± 0.37
LPS		206.05 ± 8.02	18.32 ± 0.85	10.78 ± 0.57	ND	ND	2.39 ± 0.35	1.53 ± 0.26

Abbreviations: DA: dopamine; DOPAC: dihydroxyphenylacetic acid; HVA: homovanillic acid; NE: norepinephrine; MHPG: 3-methoxy-4-hydroxyphenylglycol; 5-HT: serotonin; 5-HIAA: 5-hydroxyindoleacetic acid; ND: not detected;

* $p < 0.05$ compared to saline control.

Table 3

Concentrations of monoamines and their metabolites in the hippocampus of male C57BL/6 mice treated with vehicle (saline) or LPS (0.25 mg/kg BW; i.p., twice weekly; $n = 5$ per group) in 13, 25 and 13-week on + 12-week off groups. Data are presented as mean $\pm$ SEM; unit: ng/mg protein.

Brain neurotransmitters and neurotransmitter metabolites								
Brain region		Hippocampus						
Treatment	13-week	DA	DOPAC	HVA	NE	MHPG	5-HT	5-HIAA
Saline		0.46 ± 0.09	0.48 ± 0.28	0.23 ± 0.10	7.48 ± 0.54	6.06 ± 0.42	3.22 ± 0.20	2.84 ± 0.21
LPS		0.28 ± 0.05	0.19 ± 0.02	0.22 ± 0.10	7.42 ± 0.76	6.47 ± 0.50	2.77 ± 0.16	2.48 ± 0.13
25-week								
Saline		0.93 ± 0.35	2.77 ± 0.13	0.18 ± 0.03	10.69 ± 1.13	11.81 ± 0.58	3.63 ± 0.78	3.25 ± 0.28
LPS		1.13 ± 0.19	2.67 ± 0.19	0.37 ± 0.12	10.48 ± 0.48	11.33 ± 0.53	3.70 ± 0.73	3.31 ± 0.28
13-week on + 12-week off								
Saline		0.87 ± 0.13	2.72 ± 0.22	0.32 ± 0.07	10.34 ± 0.53	11.74 ± 0.92	3.43 ± 0.48	3.01 ± 0.37
LPS		1.07 ± 0.06	3.15 ± 0.38	0.25 ± 0.10	9.90 ± 0.74	13.67 ± 1.88	2.41 ± 0.60	2.36 ± 0.64

Abbreviations: DA: dopamine; DOPAC: dihydroxyphenylacetic acid; HVA: homovanillic acid; NE: norepinephrine; MHPG: 3-methoxy-4-hydroxyphenylglycol; 5-HT: serotonin; 5-HIAA: 5-hydroxyindoleacetic acid.

* $p < 0.05$ compared to saline control.