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Short-term Effects of an Endotoxin on Substantia Nigra Dopamine Neurons

Kaela R.S. Reinert, Claudia D. Umphlet, Ariana Quattlebaum, and Heather A. Boger*

Department of Neurosciences and Center on Aging, Medical University of South Carolina, 173 Ashley Ave, BSB Suite 403, MSC 510, Charleston, SC, 29425, USA

Abstract

Inflammation has been implicated in the pathology of several neurodegenerative diseases, including Parkinson's disease (PD). Studies using the endotoxin lipopolysaccharide (LPS), a potent inflammogen, show that systemic insults can trigger prolonged microglial activation and pro-inflammatory cytokine production leading to degeneration of substantia nigra (SN) dopamine (DA) neurons, mimicking idiopathic PD. Because rapid effects of LPS on SN neurons had not been investigated previously, the focus of this study is to assess time-dependent alterations in SN neuroinflammation, DAergic neurons, and neuronal signaling cascades following LPS administration. LPS (5 mg/kg, i.p.) or saline (0.9% NaCl) was administered to 8-month-old male mice. At 3hrs, 5hrs, and 12hrs post-injection, the morphology of the SN was assessed using antibodies directed against tyrosine hydroxylase (TH, DAergic marker), Iba-1 (pan-microglial marker), phospho-ERK, and phospho-CREB (signaling). LPS administration significantly reduced TH-immunoreactivity (ir) at all time-points with the greatest reduction observed at 12 h post-injection. Reduced TH-ir was accompanied by a significant increase in activated microglia at all time-points following LPS. By 12 h post-injection, LPS-treated mice exhibited activated as well as reactive microglia, which can result in neuronal damage. These data demonstrate that the initial reduction in TH-ir observed after an LPS injection was not concomitant with morphological alterations in microglial cells, even though a significant increase in phospho-ERK was observed in glial cells as soon as 3 h post-injection. It is possible that the initial alteration in DA phenotype (TH reduction) may perpetuate an inflammatory response that persists and leads to further DAergic damage.

Keywords

Lipopolysaccharide; Dopamine; Inflammation; Substantia nigra

1. Introduction

Inflammation is an active defense reaction of multi-cellular organisms against diverse insults, designed to remove or inactivate noxious agents and to inhibit and reverse their detrimental effects (for review see Gao et al., 2003, Liu et al., 2003). Microglia are responsible for producing an inflammatory reaction to insults (Streit et al., 2004). Normally, microglia are in a resting state characterized by a ramified morphology, and low expression

*Corresponding author. Tel.: +18438762230; fax: 18437924423. boger@musc.edu.

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levels of MHC Class II elements. However, during inflammatory changes in their micro-environment or as a consequence of pathological changes, they transform into their activated state by displaying an amoeboid morphology and increased thickness and number of processes (Kreutzberg, 1996).

Microglial activation occurs in association with various brain injuries (Kato et al., 2000), including damage common to neurological diseases (De Simone et al., 1995; Banati et al., 1998; Versijpt et al., 2003), and following administration of neurotoxins utilized to model neurodegenerative disorders (Jorgensen et al., 1993; Scali et al., 1999; Fiedorowicz et al., 2001). Microglia perform various functions in tissue repair and neural regeneration by releasing trophic factors (Nakajima and Kohsaka, 1993). However, activated microglia mainly act as scavenger cells by initiating inflammation and exacerbating degeneration causing an increased release of pro-inflammatory molecules such as reactive oxygen species (ROS), interleukin (IL)-1 β , tumor necrosis factor α (TNF α), and nitric oxide (NO), the overproduction of which can cause toxicity (Kennedy et al., 1997; Hald et al., 2005).

Direct evidence of cell loss triggered by microglial activation comes from studies assessing *in vivo* effects of the gram-negative bacterial cell wall component lipopolysaccharide (LPS). LPS has been injected into the SN of rats causing microglial activation and degeneration of the dopaminergic system (Castano et al., 1998; Herrera et al., 2005). Similarly, a single systemic LPS injection in mice models has been shown to induce chronic neuroinflammation with subsequent neurodegeneration (Qin et al., 2007; Granholm et al., 2011). *In vitro* studies using mixed neuron-glia cultures treated with LPS demonstrated rapid microglial activation followed by neuronal degeneration (Gao et al., 2002). Additionally, LPS administration *in vivo* has been shown to result in reduced SN TH-immunoreactivity as well as DA content when administered either prenatally or postnatally in both mice (Liu et al., 2008; Granholm et al., 2011) and rat studies (Castano et al., 1998). These studies suggested that SN DA neurons may be particularly sensitive to inflammatory processes in the brain, but studies have not been undertaken to determine the temporal determinants of LPS-induced dopaminergic degeneration. Therefore, the aims of the current study were to examine short-term effects of systemic LPS injections on DA neuron degeneration, microglial activation, and signaling molecules associated with microglial activation.

2. Results

2.1 Rapid Effects of LPS on Substantia Nigra Dopamine Neurons

The effects of systemic LPS injections during the time-points assessed did not include any morphological changes in the striatum, and therefore appeared to be restricted to the SN. Evaluation of TH+ immunoreactivity density, which indicates DAergic neurite density, in the dorsal striatum enabled quantitative analysis of TH-ir in the striatum at 3, 5, and 12 hrs post-injection, and revealed no differences between the various time-points or when compared to saline-treated controls (data not presented).

As can be seen from Figure 1 and as analyzed using a one-way ANOVA, LPS administration resulted in reduced TH-ir in the SN compared to saline controls, regardless of time following injection ($F(3,19)=9.67$, $p<0.05$). At 3 and 5 hrs post-injection, LPS treatment resulted in a significant reduction of SN TH-ir compared to saline treatment as revealed with Bonferroni's posthoc analysis (Figure 1A, B, C, H; $p<0.05$). At 3 and 5 hrs post-LPS administration, the TH-positive cells began to display nuclear swelling, accompanied by punctuate processes, suggesting an acute degenerative process (Figure 1 E–G). Nigral TH expression did not differ between the 3 and 5 hrs post-LPS injection; however, between 5- and 12-hours following administration, TH-ir was further reduced

(Figure 1C, D, H; $p < 0.01$). Cells still expressing TH at 12 hours post-injection were fewer in numbers and demonstrated a reduction in size, indicative of degeneration.

2.2 Acute effects of LPS on Microglial Activation

Densitometry measurements of Iba-1-immunoreactivity (ir) indicative of microglial activation revealed a significant effect of Treatment after one-way ANOVA analysis ($F(3,19)=7.314$, $p < 0.05$). Bonferroni's posthoc analysis indicated that at 3 hrs post-LPS injection, Iba-ir was significantly elevated compared to saline treated mice (Figure 2A, B, G, $p < 0.01$). At 5 hrs post-LPS injection, there was further increase in Iba-ir, which appeared to plateau between 5 and 12 hrs post-LPS, when compared to saline treated mice (Figure 2C, D, G, $p < 0.001$). Between 3 and 5 hrs post-injection, a majority of the microglial cells in the SN exhibited a change in morphological appearance (Figure 2E, F). The cells went from a "resting" state, indicated by a smaller cell body and longer processes, to an "activated" state in which the cell bodies were enlarged and the processes were shorter and thicker. The density of Iba-1-ir was still significantly elevated compared to saline-treated mice and LPS-treated mice at 12 hours post-injection, indicating an increase in Iba-1 expression within each cell and/or increased proliferation of this cell type in the SN as a result of the LPS injection.

2.3 High-Dose LPS Induces Microglial ERK/MAPK Signaling Molecules

Because an increase in microglial activation was observed 3 hrs after a high-dose LPS administration (Figure 2A, B, G), phospho-ERK immunoreactivity potentially indicative of microglial cellular signaling was assessed in the SN (Figure 3). Statistical analysis utilizing a one-way ANOVA showed a significant increase in phospho-ERK-ir in LPS treated mice, regardless of time-point ($F(3,19)=10.042$, $p < 0.001$), however, no difference was observed between the LPS time-points (Figure 3B, C, D, E). At 5 hrs post-injection, phospho-ERK-ir could be seen not only in cell bodies, but in the processes as well (see inset in Figure 3C). The p-ERK-ir cells in the SN region closely resembled microglial cells morphologically, and increased p-ERK IR was continually observed through the 12 hour timepoint.

The increase of phosphorylated ERK in the mice treated with LPS was accompanied by a significant increase in phospho-CREB-ir as a result of LPS treatment ($F(3,19)=9.64$, $p < 0.05$). Phospho-CREB-ir was significantly increased at 3- and 5-hrs post-injection (Figure 4A, B, C, E; $p < 0.05$). As indicated by quantification, phospho-CREB-ir peaked at 5 hrs ($p < 0.001$) and then returned to levels comparable to saline-injected mice by 12 hrs post-injection (Figure 4A, D, E). As can be seen from the Nissl counterstain, greater phospho-CREB is observed in neuronal populations within the SN (Figure 4F, arrows). Taken together, these data indicate early transient changes of cellular signaling molecules that differ between glial cells and neuronal populations within the SN.

3. Discussion

This is the first study to demonstrate the short-term effects of a high-dose LPS administration in vivo on the SN DAergic system. The focus of this study was to assess time-dependent alterations in neuroinflammation in the SN. To accomplish this, we assessed the SN of male C57Bl/6J mice with antibodies directed against TH, Iba-1, phospho-ERK, and phospho-CREB at 3, 5, and 12 hrs following a systemic LPS (5mg/kg) or saline injection.

LPS has been used repeatedly in rat and mouse animal models to determine the role of inflammation on the degeneration of SN DAergic neurons as these DAergic neurons have been shown to have increased vulnerability to processes such as inflammation and oxidative

stress (Liu et al., 2008). Previous studies have demonstrated that a single injection of LPS results in a delayed and progressive loss of TH-immunoreactive neurons in C57BL/6 mice and that this loss was mediated through pro-inflammatory cytokines entry into the CNS (Ling et al., 2002; 2006). However, based on our current study, we have been able to determine that there is an initial significant decline in DAergic SN neurons as well as a significant increase in neuronal phospho-CREB, as early as 3hrs post-LPS injection, which is followed by yet another significant decline in TH expression and the presence of apoptotic neuronal bodies between 5 hrs and 12 hrs post-LPS injection. Additionally, we saw that microglia had migrated into the damaged region by the 3hr post-injection time-point, and underwent greatest activation at the 5hr post-LPS injection time-point. This correlated with the phosphorylation of ERK within the microglia by the 3hr post-LPS injection time-point because phosphorylated ERK is associated with the migration of microglia. Also, the presence of phospho-ERK within the processes of the microglia correlates with the peaking of microglial activation at the 5hr post-LPS-injection time-point. After the peak of microglial activation at the 5hr post-LPS-injection time-point, there is the presence of apoptotic DAergic cell bodies in the SN at the 12 hrs post-LPS-injection time-point. The culmination of these data suggests that the initial DAergic neuronal loss is not directly related to the neuroinflammatory properties of LPS, rather the LPS insult itself. Furthermore, the initial DAergic loss results in an inflammatory response that persists and leads to further DAergic damage. These results warrant future studies to look at more immediate time-points following this high-dose LPS administration to determine if this immediate response of a loss of TH-immunoreactivity is a direct result of LPS administration.

The production of anti- and pro-inflammatory cytokines by activated microglia may serve as a possible explanation for these data. During the inflammatory response to the DAergic phenotypic loss, the microglia may initially produce anti-inflammatory cytokines, which enable the DAergic neuronal cells to synthesize the necessary proteins to survive the insult of LPS (Qian et al. 2006). This could explain why the level of TH expression remains consistent for the 3hr and 5hr post-LPS-injection time-points. However, between the 5 and 12 hrs LPS time-points, it is possible that microglia begin produce pro-inflammatory cytokines instead. These pro-inflammatory cytokines cause injury to different areas of the neuronal cell bodies, including the mitochondria, resulting in apoptosis (Hunter et al. 2009). Thus, by the 12hr post-LPS-injection time-point, we see remnants of SN DA neurons as well as reduced phosphorylation of the transcription factor, CREB. These data warrant further studies to determine the direct mechanism by which microglia play in the immediate degeneration of DAergic neurons that occurs as a result of this high-dose LPS administration *in vivo*, such as determining which pro- vs. anti-inflammatory cytokines are produced.

Future research should focus on the time-dependent production of anti- and pro-inflammatory cytokines by activated microglia. If activated microglia are changing from primarily anti-inflammatory cytokine production to pro-inflammatory cytokine production as hypothesized and causing further DAergic cell death, we may then move forward and research preventative treatments for Parkinson's disease using this valuable information.

4. Experimental Procedure

4.1 Animals

Male C57BL/6J mice (8 months of age) were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were individually housed and maintained in a temperature- and humidity-controlled AAALAC accredited animal facility under a 12 hour light:dark cycle. Mice had free access to food and water at all times during experimental procedures. All experimental protocols were approved by the Institutional Animal Care and Use Committee

at the Medical University of South Carolina and were consistent with the guidelines of the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1996). Mice were acclimated to the vivarium for a minimum of one week prior to the start of the experiments.

4.2 Experimental Design

Following the one-week acclimation period, the mice were randomly assigned to the various treatment groups. The mice were administered either physiological saline (0.9% NaCl) or LPS (O111:B4; 5mg/kg, i.p., Sigma Inc., St. Louis, MO, USA), N=6 per group. The mice were then anesthetized and perfused at 3, 5, or 12 hrs post-injection for immunohistochemical assessment.

4.3 Immunohistochemistry

The mice were anesthetized deeply using isoflurane and perfused transcardially with saline followed by 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.4) for immunohistochemical analysis. The brains were removed, postfixed in 4% paraformaldehyde for 24 hrs, and then transferred to 30% sucrose in 0.1 M PBS for at least 24 hrs before sectioning for histochemical analysis, according to our standard protocol (see e.g. Granholm et al., 1997a, b; Boger et al., 2006). The striatum and midbrain were sectioned on a cryostat (Microm, Zeiss, Thornwood, NY, USA) at 45 μ m. Serial sections through the SN (every 6th section) were processed for free-floating immunohistochemistry using a rabbit polyclonal antibody against tyrosine hydroxylase (TH; 1:1000; Pel-Freeze Biologicals, Inc., Roger, AZ, USA), a rabbit polyclonal antibody against Iba-1 (pan marker for microglia; 1:1,000, Wako Pure Chemical Industries Ltd, Richmond, VI.), a rabbit polyclonal antibody against phospho-ERK (1:500, Cell Signaling), or a rabbit polyclonal antibody against phospho-CREB (1:500, Cell Signaling). Immunodetection was performed using the avidin-biotin-immunoperoxidase method (Granholm et al., 1997a, b; Boger et al., 2006). Briefly, after a 5 min. pretreatment with 2% Triton-X-100 to allow cellular penetration of the antibodies, a subset of sections from each mouse was incubated in the primary antisera against TH, Iba-1, phospho-ERK, or phospho-CREB for 24 hrs at 4°C. The sections were then rinsed and incubated for 1hr in biotin-conjugated goat anti-rabbit secondary IgG, rinsed, and incubated for 1hr with avidin-biotin-peroxidase reagents (Elite Vectastain kit, Vector Labs, Burlingame, CA). Diaminobenzidine (DAB, Sigma) was used as a chromagen to develop the reaction using 0.05% of 3% H₂O₂ and nickel ammonium sulfate (2.5%, Sigma) was used to enhance the reaction. Each of the above steps was separated by 3×10 min. washes in PBS. Sections were mounted on glass slides and coverslipped with DPX (Sigma-Aldrich).

4.4 Semi-quantitation of immunostaining

We performed densitometry measurements on every 6th rostral-caudal section for TH-immunoreactivity (TH-ir), Iba-1-ir, phospho-ERK-ir, and phospho-CREB-ir in the SNpc at a magnification of 10× using the NIH Image program as described previously (Boger et al., 2007). For measurements of in the SNpc, an oval with area 1000 μ m×200 μ m was utilized starting on a random nigral section approximately -2.92mm relative to Bregma (Paxinos and Franklin, 2001). Measurements were thereafter performed on every 6th section through the SNpc. The hippocampus and mammillary nucleus were used as landmarks in the rostro-caudal plane, and the lateral boundary of the VTA (A10) was used as the medial landmark. Further, the oval excluded the SNpr ventrally, so that the cell body region of the SN was exclusively investigated for immunoreactivity. The span of the sectioned tissues exceeded the rostral and caudal extents of the SN, as determined by TH immunostaining on adjacent section series, allowing a systematic random design for the study with a random start of the first section. Following placement of the density template, background was subtracted and

the LUT scale was adjusted using density slicing. This approach captures all signal intensity above a threshold density and interactively discriminates them from density values below the threshold. The mean density and number of pixels per area were measured in the selected striatal and SNpc areas independently from 5 sections per mouse. The measurements were expressed as integrated density (number of pixels per area $\times$ mean density).

4.5 Statistical Analysis

Data for all immunohistochemical markers were analyzed between two groups using student's unpaired t-test via Statview. For multiple comparisons of groups, data was analyzed using one-way ANOVA comparisons, followed by Bonferroni's posthoc analysis. Significance is reported as $p < 0.05$.

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BIBLIOGRAPHY AND REFERENCES CITED

- Banati RB, Daniel SE, Blunt SB. Glial pathology but absence of apoptotic nigral neurons in long-standing Parkinson's disease. *Mov Disord.* 1998; 13(2):221–227. [PubMed: 9539333]
- Boger HA, Middaugh LD, Huang P, Zaman V, Smith AC, Hoffer BJ, Tomac AC, Granholm AC. A partial GDNF depletion leads to earlier age-related deterioration of motor function and tyrosine hydroxylase expression in the substantia nigra. *Exp Neurol.* 2006; 202(2):336–347. [PubMed: 16889771]
- Boger HA, Middaugh LD, Patrick KS, Ramamoorthy S, Denehy ED, Zhu H, Pacchioni AM, Granholm AC, McGinty JF. Long-term consequences of methamphetamine exposure in young adults are exacerbated in glial cell line-derived neurotrophic factor heterozygous mice. *J Neurosci.* 2007; 27(33):8816–8825. [PubMed: 17699663]
- Castano A, Herrera AJ, Cano J, Machado A. Lipopolysaccharide intranigral injection induces inflammatory reaction and damage in nigrostriatal dopaminergic system. *Journal of Neurochemistry.* 1998; 70(4):1584–1592. [PubMed: 9580157]
- Fiedorowicz A, Figiel I, Kami ska B, Zaremba M, Wilk S, Oderfeld-Nowak B. Dentate granule neuron apoptosis and glia activation in murine hippocampus induced by trimethyltin exposure. *Brain Res.* 2001; 912(2):116–127. [PubMed: 11532427]
- Finsen BR, Jørgensen MB, Diemer NH, Zimmer J. Microglial MHC antigen expression after ischemic and kainic acid lesions of the adult rat hippocampus. *Glia.* 1993; 7(1):41–49. [PubMed: 8423061]
- Franciosi S, Ryu JK, Shim Y, Hill A, Connolly C, Hayden MR, McLarnon JG, Leavitt BR. Age-dependent neurovascular abnormalities and altered microglial morphology in the YAC128 mouse model of Huntington disease. *Neurobiol Dis.* 2012; 45(1):438–449. [PubMed: 21946335]
- Gao HM, Jiang J, Wilson B, Zhang W, Hong JS, Liu B. Microglial activation-mediated delayed and progressive degeneration of rat nigral dopaminergic neurons: relevance to Parkinson's disease. *Journal of Neurochemistry.* 2002; 81(6):1285–1297. [PubMed: 12068076]
- Giovannini MG, Scali C, Prosperi C, Bellucci A, Vannucchi MG, Rosi S, Pepeu G, Casamenti F. Beta-amyloid-induced inflammation and cholinergic hypofunction in the rat brain in vivo: involvement of the p38MAPK pathway. *Neurobiol Dis.* 2002; 11(2):257–274. [PubMed: 12505419]
- Graeber MB, Streit WJ. Microglia: biology and pathology. *Acta Neuropathol.* 2010; 119(1):89–105. Review. [PubMed: 20012873]
- Granholm AC, Zaman V, Godbee J, Smith M, Ramadan R, Umphlet C, Randall P, Bhat NR, Rohrer B, Middaugh LD, Boger HA. Prenatal LPS increases inflammation in the substantia nigra of Gdnf heterozygous mice. *Brain Pathol.* 2011; 21(3):330–348. [PubMed: 20969653]
- Hald A, Lotharius J. Oxidative stress and inflammation in Parkinson's disease: is there a causal link? *Exp Neurol.* 2005; 193(2):279–290. Review. [PubMed: 15869932]

- Herrera AJ, Tomás-Camardiel M, Venero JL, Cano J, Machado A. Inflammatory process as a determinant factor for the degeneration of substantia nigra dopaminergic neurons. *J Neural Transm.* 2005; 112(1):111–119. Review. [PubMed: 15599609]
- Hunter RL, Baohua C, Dong-Young Choi, Lui M, Liu S, Cass WA, Bing G. Intrastriatal lipopolysaccharide injection induces Parkinsonism in C57/B6 mice. *J Neurosci Res.* 2009; 87(8): 1913–1921. [PubMed: 19224579]
- Kato H, Walz W. The initiation of the microglial response. *Brain Pathol.* 2000; 10(1):137–143. Review. [PubMed: 10668903]
- Liu YQL, Wilson B, Wu X, Qian L, Granholm AC, Crews FT, Hong JS. Endotoxin induces a delayed loss of TH-IR neurons in substantia nigra and motor behavioral deficits. *Neurotoxicology.* 2008; 29(5):864–870. [PubMed: 18471886]
- Mogi M, Harada M, Kondo T, Riederer P, Inagaki H, Minami M, Nagatsu T. Interleukin-1 beta, interleukin-6, epidermal growth factor and transforming growth factor-alpha are elevated in the brain from parkinsonian patients. *Neurosci Lett.* 1994; 180(2):147–150. [PubMed: 7700568]
- Nagatsu T, Mogi M, Ichinose H, Togari A. Changes in cytokines and neurotrophins in Parkinson's disease. *J Neural Transm Suppl.* 2000; (60):277–290. [PubMed: 11205147]
- Nakajima K, Kohsaka S. Microglia: neuroprotective and neurotrophic cells in the central nervous system. *Curr Drug Targets Cardiovasc Haematol Disord.* 2004; 4(1):65–84. Review. [PubMed: 15032653]
- Qian L, Block ML, Wei SJ, Lin C, Reece J, Pang H, Wilson B, Hong JS, Flood PM. Interleukin-10 protects lipopolysaccharide-induced neurotoxicity in primary midbrain cultures by inhibiting the function of NADPH oxidase. *J Pharmacol Exp Ther.* 2006; 319:44–52. [PubMed: 16807359]
- Romero-Sandoval EA, Horvath R, Landry RP, DeLeo JA. Cannabinoid receptor type 2 activation induces a microglial anti-inflammatory phenotype and reduces migration via MKP induction and ERK dephosphorylation. *Mol Pain.* 2009; 5:25. [PubMed: 19476641]
- Streit WJ, Mrak RE, Griffin WS. Microglia and neuroinflammation: a pathological perspective. *J Neuroinflammation.* 2004; 1(1):14. [PubMed: 15285801]
- Versijpt JJ, Dumont F, Van Laere KJ, Decoo D, Santens P, Audenaert K, Achten E, Slegers G, Dierckx RA, Korf J. Assessment of neuroinflammation and microglial activation in Alzheimer's disease with radiolabelled PK11195 and single photon emission computed tomography. A pilot study. *Eur Neurol.* 2003; 50(1):39–47. [PubMed: 12824711]
- Qin L, Wu X, Block ML, Liu Y, Breese G, Hong JS, Knapp DJ, Crews FT. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia.* 2007; 55(5):453–462. [PubMed: 17203472]
- Zhuang ZY, Gerner P, Woolf CJ, Ji RR. ERK is sequentially activated in neurons, microglia, and astrocytes by spinal nerve ligation and contributes to mechanical allodynia in this neuropathic pain model. *Pain.* 2005; 114(1–2):149–159. [PubMed: 15733640]

Highlights

- We examine the short-term effects of LPS administration on the substantia nigra of mice.
- LPS administration resulted in a time-dependent increase in microglia activation.
- LPS administration resulted in altered phosphorylated ERK and CREB labeling over time in microglia and neurons, respectively.
- High-dose LPS resulted in a significant reduction of tyrosine-hydroxylase labeling over a 12-hour time period.

Figure 1.

Nigral TH-ir is reduced following LPS administration. Photomicrographs of TH-ir in the SN of (A) saline-treated mice, (B) LPS-treated mice at 3 hrs, (C) LPS-treated mice at 5 hrs, and (D) LPS-treated mice at 12 hrs (magnification=10×). (E–G) Treatment groups at magnification=60×. (H) Quantification of the average TH-ir density in the SNpc. LPS-treatment, regardless of time-point, resulted in reduced TH-ir compared to saline-treatment ($p<0.05$). No difference existed in the density of TH-ir between 3- and 5-hr LPS treated mice, however, by 12 hrs, TH-ir was significantly reduced compared to 3- and 5-hrs post-injection ($p<0.01$). At 5-hrs post-LPS injection, nuclear swelling was evident as indicated by the large arrow. By 12 hrs post-LPS injection, existing TH-ir cells are showing changes in morphology, including cellular shrinking as indicated by the large arrows and punctate processes as indicated by the thin arrow in 1F. SNpc outlined by straight lines, SNpr outlined by oval shape. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Figure 2.

Increased microglia in the SN after LPS treatment. Microglia are shown in the SN by Iba-1 labeling in (A) saline-treated mice, (B) LPS-treated mice at 3 hrs, (C) LPS-treated mice at 5 hrs, and (D) LPS-treated mice at 12 hrs (magnification 20 $\times$). (E-F) LPS-treated mice at 3- and 5-hrs at magnification =60 $\times$. (G) Quantification of the average Iba-1-ir density in the SN of the treatment groups. Iba-1-ir in the SN was significantly increased following LPS treatment compared to saline-treated mice ($p < 0.05$). This increase in Iba-1 labeling is indicated by microglial cell body enlargement as well as a thickening of the processes (E, F). ** $p < 0.01$, *** $p < 0.001$.

Figure 3.

LPS administration resulted in increased glial phospho-ERK expression. Phospho-ERK-ir measured in the SN of (A) saline-treated mice, (B) LPS-treated mice at 3 hrs, (C) LPS-treated mice at 5 hrs, and (D) LPS-treated mice at 12 hrs (magnification 40 $\times$). (E) Quantification of phospho-ERK-ir in the SN following LPS injections. LPS treatment resulted in a significant increase of phospho-ERK-ir in the SN, regardless of time point (* $p < 0.001$). This increase of labeling appeared to be within glial cells as indicated by morphological appearance (inset, B, magnification=60 $\times$).

Figure 4.

Increased nigral phospho-CREB-ir following LPS administration. Phospho-CREB-ir as measured in (A) saline-treated mice, (B) (B) LPS-treated mice at 3 hrs, (C) LPS-treated mice at 5 hrs, and (D) LPS-treated mice at 12 hrs (magnification 40 $\times$). (E) Quantification of phospho-CREB-ir in the SN following LPS injections. (F) Nissl co-labeling (blue) with phosphor-CREB-ir (brown) in the SN as indicated by arrows (magnification=60 $\times$). LPS injections resulted in a significant increase in SN phospho-CREB labeling at 3 hrs ($p<0.01$) and 5 hrs ($p<0.001$). Phospho-CREB-ir peaked at 5 hrs and then returned to comparable levels of saline-treated mice by 12 hrs post-LPS injection. Nissl co-labeling confirmed the localization of phospho-CREB to neuronal populations (F). * $p<0.05$, ** $p<0.01$, *** $p<0.001$.