Age and duration of inflammatory environment differentially affect the neuroimmune response and catecholaminergic neurons in the midbrain and brainstem

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1. Introduction

Activation of the brain’s resident microglia occurs during normal aging, is associated with many neurodegenerative diseases such as Parkinson’s disease (PD) and Alzheimer’s disease (AD), and may drive a self-propagating toxic cycle promoted by the release of pro-inflammatory and loss of protective mediators (Aarsland et al., 2001; Akiyama et al., 2000; Bartels and Leenders, 2005; Block and Hong, 2005; Cobbs et al., 2012; Griffin et al., 1989; Hobson and Meara, 2004; Hughes et al., 2000; Swardfager et al., 2010; Whitton, 2007). When these processes are triggered within vulnerable brain regions, they may lead to the loss of acetylcholinergic neurons in the nucleus basalis magnocellularis (Whitton, 2007; Willard et al., 1999) as well as dopaminergic neurons in the substantia nigra pars compacta (SNpc), noradrenergic (NE) neurons in the locus coeruleus (LC) and all regions that show significant early cell loss in the brains of patients with PD and AD (Braak et al., 2003; Grudzien et al., 2007; Halliday et al., 2006; Rudow et al., 2008; Szot et al., 2006).

We and others have speculated that the consequences of neuroinflammation associated with microglial activation, are carefully regulated until, because of normal aging or the deposition of toxic proteins, there is a gradual shift to a nonequilibrium state that is permissive for neurodegenerative processes (Block and Hong, 2005; Colton and Wilcock, 2010; Smith et al., 2012; Wenk and Hauss-Wegrzyniak, 2001). Microglia can assume various phenotypes that are associated either with the release of potentially destructive, pro-inflammatory cytokines, and other toxic molecules or the expression of a cytokine profile that sustains repair, recovery, and growth. Microglia in various states of activation are detectable many years before the onset of neuropathological changes (Cagnin et al., 2006; Gerhard et al., 2006; Imamura et al., 2003). Because vulnerable brain regions are likely exposed for many decades to a complex combination of microglia in various activation states (Bilbo, 2010; Eikelenboom et al., 2010; Heneka et al., 2010; Herrup, 2010). The present study investigated the differential influence of
brain age and the duration of the pro-inflammatory environment upon the expression of pro- and anti-inflammatory genes and proteins as well as the number of activated microglia and the integrity and density of ascending catecholaminergic neural systems originating in the LC and SNpc.

2. Methods

2.1. Experimental design

Young (3 months), middle-aged (9 months), and aged (23 months) male F-344 rats (Harlan Sprague–Dawley) received chronic infusion of lipopolysaccharide (LPS) or its vehicle (artificial cerebral spinal fluid; aCSF) into the fourth ventricle for 21 or 56 days. We believe that this approach best represents the situation present during the early stages of many chronic neurodegenerative diseases. Multiple counter-balanced iterations of this study were performed to produce a total of 132 rats; yielding experimental groups with a minimum of 11 rats that were divided between biochemical (minimum 6 rats/group) and histologic (minimum 5 rats/group) analysis. Midbrain and/or brainstem regions were evaluated for protein and messenger RNA (mRNA) expression of inflammatory markers and the LPS receptor (toll-like receptor 4; TLR4), as well as the presence of MHC II-IR microglia, which was used to define activated microglia. Changes in these immune factors at 3 ages and after short (21 days) or long (56 days) of continuous LPS infusion were then evaluated with respect to changes in neurotransmitter systems including expression of genes involved in the regulation of glutamate (glutamate transporter 1; GLT1, and the cystine-glutamate anti-porter; Xct), and gene and histologic expression of the enzymes responsible for production of dopamine (tyrosine hydroxylase; TH) and norepinephrine (dopamine-ß-hydroxylase; DBH). DBH immunostaining was examined to determine specifically the integrity of norepinephrine innervation of the dentate gyrus region of the hippocampus, which does not receive a dense dopaminergic input (Gasbarri et al., 1994). TH immunostaining was used to define specifically the norepinephrine neurons in the LC and dopaminergic neurons in the SNpc.

2.2. Subjects

Rats were maintained on a 12/12-hours light–dark cycle with lights off at 09:00 in a temperature-controlled room (22 °C) with free access to food and water. All rats were sacrificed during the dark phase of the diurnal cycle. Body weights and general health were closely monitored throughout the study. All rats were allowed at least 1 week to adapt to their new environment before surgery. The experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996, and formal approval to conduct the experiments was obtained from the animal subjects review board from The Ohio State University.

2.3. Surgery

aCSF (140 mM NaCl, 3.0 mM KCl, 2.5 mM CaCl2, 1.0 mM MgCl2, and 1.2 mM Na2HPO4 adjusted to pH 7.4) or LPS (0.25 mg/h, 1.66 mg/mL prepared in aCSF; Escherichia coli, serotype 055:B5, TCA extraction, Sigma-Aldrich, St. Louis, MO, USA) were continuously infused via a cannula implanted into the fourth ventricle (–2.5 mm anterior–posterior and –7.0 mm dorsal–ventral, relative to lambda) and attached (via Tygon tubing, 0.06 O.D.) to an osmotic minipump (Alzet model #2006, to deliver 0.15 μl/h; DURECT Corporation, Cupertino, CA, USA) as previously described (Hausse-Wegrzyniak et al., 1998; Marchalant et al., 2007; Rosi et al., 2004). The average fill volume and release rates for the pump allows for an infusion up to 56 days. Postoperative care included lidocaine 1% solution applied to the exposed skin upon closure, 2 mL of isotonic saline by subcutaneous injection to prevent dehydration during recovery and 2% tylenol in the drinking water for 3 days before and after surgery.

2.4. Tissue collection

Rats used for protein and mRNA analysis were briefly anesthetized and then rapidly decapitated; their midbrain and/or brainstem (extending from just rostral to the SNpc and just caudal to the LC) and entire hippocampus were quickly dissected on ice and stored at −80 °C until processed. Blood was collected during the rapid decapitation procedure. After centrifugation at 4 °C for 15 minutes at 2500 x g, serum was collected and assayed. Rats used for immunohistochemistry were deeply anesthetized with isoflurane for a transcardiac perfusion with 80 mL of cold 0.9% saline containing 50 U/mL heparin, followed by 120 mL of 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS), pH 7.4, at a rate of 10 mL/min. Brains were postfixed overnight in the same fixative and then stored in PBS.

2.5. Protein analysis

Brainstem or serum levels of tumor necrosis factor (TNF)-α, interleukin-1 (IL-1)–α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 IL-13, IL-18 (interferon-γ inducing factor), interferon-γ, and granulocyte-macrophage-stimulating factor (GM-CSF) were quantified simultaneously with a magnetic bead-based immunoassay (Bio-Rad, BioPlex Pro Rat Standard, 171-K1002M), according to the manufacturer’s protocol. Briefly, total protein was extracted from frozen midbrain and/or brainstem with a BioPlex Cell Lysis Kit (BioRad, Richmond, CA, USA). A mixture of distinct capture beads (fluorescently dyed microspheres) each with a specific spectral address and conjugated to an antibody against one of the cytokines listed previously were dispensed across a 96-well plate and protected from light. Samples and antigen standards were added in duplicate and the plate was shaken (700 RPM, 1 hour). Then a mixture of biotinylated detection antibodies directed against each of the primary antibodies was added and the plate was shaken (700 RPM, 30 minutes); unbound materials were washed away (3 times) (BioRad, BioPlex Pro wash station). Each well was then incubated with a streptavidin–phycoerythrin conjugate reporter dye that binds to the detection antibody (700 RPM, 10 minutes); unbound materials were washed away (3 times). Each well was then suspended in assay buffer and shaken (1100 RPM for 30 seconds). Finally, the contents were passed through a dual detection multiplexing machine (Bio-Rad MAGPIX multiplex reader) with a classification algorithm that distinguishes each of the proteins by color of its bound antigen–specific bead and a reporter laser that quantifies each molecule based upon the fluorescence of bound antigen–specific streptavidin–phycoerythrin conjugate reporter dye. Values were standardized to protein content of the homogenate obtained with a Bio–Rad protein assay (Bio–Rad), and results are reported as pg/mg protein.

2.6. Real-time polymerase chain reaction mRNA analysis

Brainstems were evaluated for mRNA expression of: TNFα, IL1β, transforming growth factor-β (TGFβ), TLR4, fractalkine receptor (CX3CR1), GLT1, Xct, brain-derived neurotrophic factor (BDNF). TH was evaluated in the midbrain and/or brainstem as a precursor to LC norepinephrine as well as SNpc dopamine, and DBH was evaluated in the hippocampus because it is a precursor to norepinephrine that can distinguish input from the LC projections. Tissues were homogenized in Trizol (Life Technologies, Carlsbad, CA, USA). Total RNA
was extracted with a NucleoSpin RNA II kit (Macherey-Nagel, Düren, Germany); 1 μg of total RNA was reverse-transcribed to create a complementary DNA library using the iScript reverse transcription Supermix for quantitative real-time polymerase chain reaction (BioRad). Primers were designed with PrimerQuest software (version 3.4 Integrated DNA Technologies, Coralville, IA, USA; Table 1), and specificity was ensured by examining primer alignments with the BLAST database. Primers and Sso Advanced SYBR Green Supermix (BioRad) were prepared with RNase-free water. For PCR amplification, mix (19°C to 100°C with the C1000 Thermal Cycler (Bio-Rad). Amplification conditions were: 95°C for 30 seconds and 40 cycles of PCR (denaturation: 95°C for 5 seconds, annealing and/or extension: 60°C for 30 seconds), followed by melting curves to verify the absence of primer dimers. Two negative controls were performed during each quantitative PCR experiment: reaction without the reverse transcription (−RT) to confirm the absence of genomic DNA contamination, and samples with no added complementary DNA template (H2O only). The cycle (Ct) at which expression levels crossed threshold was normalized to the Ct of the reference gene glyceraldehyde-3-phosphate dehydrogenase; producing ΔCt with arbitrary units of total gene expression.

2.7. Immunocytochemistry

Free-floating coronal sections (40 μm) were obtained with a vibratome (Leica, Model VT1000S) from perfused brains that were kept cold but never frozen. All reactions took place on an agitator at room temperature, except for overnight incubations, and all rinses (3 times 10 minutes) were in PBS with 0.05% tween 20. Tissues were rinsed, quenched of native peroxidase activity with 0.3% H2O2 in 50% methanol for 1 hour, rinsed, blocked for nonspecific binding in 5% normal goat serum for 1 hour, and incubated in primary antibody diluted in 5% normal goat serum overnight at 4°C. Double-immunostaining of all sections was achieved using these antibodies: anti-class II major histocompatibility complex (MHC II, 1: 200, mouse monoclonal, Pharmingen, San Diego, CA, USA), anti-class I MHC (1:200, mouse monoclonal, Pharmingen, San Diego, CA, USA), anti-tubulin (1:100 for the LC and 1:500 for the SNpc), anti-TH (1:3000 for the LC and 1:1500 for the SNpc, rabbit polyclonal, Millipore, Billerica, MA, USA #AB152). Thereafter, sections were incubated in a corresponding biotinylated secondary antibody (1:200, Vector, Burlingame, CA, USA #BA-1000, BA-2001) for 1.5 hours, rinsed, incubated for 1 hour with avidin-biotinylated horseradish peroxidase (ABC kit, Vector), rinsed, and visualized by incubation with the chromogens 0.05% 3,3-diaminobenzidine tetrahydrochloride (DAB, Vector), or 5G Blue (Vector). Double-chromogenic staining continued from this point by placing sections into another primary antibody and repeating these steps. After a final rinse, sections were mounted onto gel-coated slides and air-dried. All slides were then dehydrated with serial dilutions of ethanol and cover-slipped with Cytoseal mounting medium (Allan Scientific, Kalamazoo, MI, USA). No staining was detected in the absence of the primary or secondary antibodies. Confidence was also established for antibodies that produced a band of the correct molecular weight in western blot analysis.

Immunoreactive (IR) microglia and neurons were examined in the LC or SNpc by light microscopy (Nikon 90i with a DS-5M-L1 digital camera using Elements 3.1; Nikon Instruments, Melville, NY, USA), on an average of 10 evenly spaced slices per region. Cells were manually counted with Nikon Elements and expressed as number of IR cells per mm².

2.8. Statistical analysis

Analyses of variance were performed followed by Fishers Protected Least Significant Difference for post hoc comparisons using SigmaPlot (v.12.3 Systat, San Jose, CA, USA). Control aCSF is shown in some graphs as 1 group collapsed across infusion duration, but aCSF groups were not collapsed for statistical analysis. Results are expressed as means ± standard error of the mean, and significant differences are marked between treatment (*), infusion duration (#), and age (†). 

3. Results

3.1. Protein analysis

LPS infusion significantly increased the pro-inflammatory cytokines IL-1α (F1,45 = 19.4, p < 0.001) and IL-1ß (F1,46 = 58.9, p < 0.001)
Fig. 1. Brainstem cytokines levels and gene expression. (A) Lipopolysaccharide (LPS) exposure significantly (*p < 0.05) increased interleukin-1 beta (IL-1β) levels and a far lesser increase in IL-1α levels. The duration of the LPS infusion was independently responsible with a significant (#p < 0.001) increase in the brainstem level of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-12, IL-13, and granulocyte-macrophage-stimulating factor (GM-CSF). The age of the rat was independently responsible for an increase in interferon gamma (IFNγ) levels in the 23 months aged rats infused with LPS and for a blunting of the LPS effects on IL-1β in the 9 and 23 months aged rats (#p < 0.05). (B) LPS significantly (*p < 0.001) increased the gene expression of the fractalkine receptor, IL1β, transforming growth factor-β (TGFβ), toll-like receptor 4 (TLR4), tumor necrosis factor alpha (TNFα), and cystine-glutamate anti-exchanger.
levels in the midbrain and/or brainstem regions that included the substantia nigra and LC (Fig. 1A). However, the IL-1β response to 21 days of LPS infusion was significantly (p < 0.05) blunted in both middle-aged and aged rats. LPS infusion over 56 days significantly (all F > 24, p < 0.001) increased midbrain and/or brainstem levels of IL-1α, IL-2, IL-4, IL-5, IL-6, IL-12, IL-13, and GM-CSF compared with 21 days of LPS infusion in rats of the same age. Although aged rats infused with LPS for 21 days had a blunted IL-1β response, this group had elevated pro-inflammatory IFNγ levels compared with 21 days of LPS infusion in younger rats (F2, 48 = 3.62, p < 0.05).

Serum levels of IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, GM-CSF, INFγ, and TNFα were unchanged across all age and treatment groups (data not shown), and may indicate that intra-cranial infusion of LPS did not have a marked response on the peripheral immune response.

3.2. mRNA expression

LPS exposure significantly (all F > 32, p < 0.001) increased the gene expression of pro-inflammatory IL1β and TNFα, the LPS receptor TLR4, anti-inflammatory TGFβ and CX3CR1 as well as the glutamate-antiporter XcT (Fig. 1B). Consistent with the results observed in protein analysis, the IL-1β response to 56 days of LPS infusion was significantly (p < 0.05) blunted in both middle-aged and aged rats compared with young rats. Gene expression of TGFβ increases more after 56 days of LPS infusion than 21 days infusion in middle-aged rats (F1, 48 = 6.39, p < 0.01) and a similar duration-dependent increase in GLT1 is observed in young and middle-aged rats (F1, 48 = 12.1, p < 0.001). TGFβ is anti-inflammatory and GLT1 serves to sequester extracellular glutamate that can be excitotoxic; an increase in these factors after prolonged LPS exposure could be a protective mechanism that does not occur in aged rats. Consistent with this, aged rats infused with LPS express less of the trophic factor BDNF than younger rats infused with LPS (F1, 48 = 8.94, p < 0.001) and by middle-aged rats infused with LPS express less XcT (F1, 48 = 4.13, p < 0.05) which could otherwise be used to acquire cytsteine for anti-oxidant production. Interestingly, across all ages, 56 days of LPS infusion was responsible for a significant decrease (all F > 6, p < 0.01) in the gene expression of TH within the midbrain and/or brainstem and a corresponding elevation in DBH gene expression within the hippocampus; suggesting a loss of midbrain and/or brainstem catecholamine production capacity and a compensatory increase in hippocampal catecholamine production capacity.

3.3. Immunohistochemistry

The density of TH-IR cells in the LC, that is the number of cell profiles/sq. mm, was significantly decreased by the LPS infusion (F1, 41 = 7.44, p < 0.01, Fig. 2A vs. B) and was not dependent upon the age of the rat or the duration of the LPS exposure (p > 0.1). The density of MHC II-IR microglia (Fig. 2B and C) throughout the brainstem and particularly within the LC was significantly increased by the LPS infusion (F1, 41 = 14.3, p < 0.001) and further increased by the duration of the exposure (F1, 41 = 11.0, p < 0.01).

The density of TH-IR cells in the SNpc (Fig. 3) was significantly (F2, 42 = 5.26, p < 0.01) decreased because of aging. There was a significant main effect of duration of LPS exposure (F1, 42 = 6.78, p < 0.01); post hoc tests revealed that the number of TH-IR cells in the SNpc of young and middle-aged rats were elevated after 56 days of LPS infusion compared with 21 days infusion (p < 0.05). Our previous stereological analysis did not find evidence for tissue shrinkage (Brothers et al., 2013a). This recovery in the density of TH-IR in the SNpc was not observed in the aged rats. We have previously reported a statistically significant increase in the number of MHC II-IR microglia within the SNpc following LPS exposure that was associated with a decline in the number of phosphorylated (i.e., active) TH-IR SNpc cells (Brothers et al., 2013a).

4. Discussion

We induced a pro-inflammatory environment in the brains of young, middle-aged, and aged male rats, and then systematically documented an evolving series of biochemical and genetic changes in the midbrain and/or brainstem induced by the chronic infusion of picomolar levels of LPS into the fourth ventricle. These changes represent a complex interplay between glia and neurons in the presence of continuous stimulation of TLR4 receptors on microglia, and suggest that a prolonged pro-inflammatory environment interacts with age to reduce the capacity of ascending catecholaminergic nuclei in the midbrain and brainstem. These findings relate to the pre-symptomatic inflammatory environment observed in AD and PD and early loss of these ascending systems in disease progression (Aarsland et al., 2001; Akiyama et al., 2000; Bartels and Leenders, 2005; Block and Hong, 2005; Cribs et al., 2012; Griffin et al., 1989; Hobson and Meara, 2004; Hughes et al., 2000; Swardfager et al., 2010; Whitton, 2007).

LPS was used as a chemical tool to stimulate the LTR4/CD14 complex expressed by microglia; administered as a continuous low dose, LPS was not sufficient to produce any peripheral manifestations of infectious disease processes (such as elevated serum levels of inflammatory cytokines) or down-regulation in microglial TLR4 gene expression in the brain (Bardou et al., 2013; Brothers et al., 2013b). LPS did promote a central pro-inflammatory environment. For example, LPS increased midbrain and/or brainstem levels of pro-inflammatory IL-1β and IL-1α. IL-1β gene and protein levels correlated significantly with each other (r = 0.74, p < 0.001). This variance in response to LPS is likely because of the differences in post-translational control of IL-1β protein production (Chen et al., 2006). This distinction may be important, given the role of IL-1β in the degeneration of SNpc dopaminergic neurons in PD (Koprich et al., 2008). Interestingly, elevations in IL-1β gene and protein expression because of LPS infusion were blunted in both middle-aged and aged rats. Ordinarily, GM-CSF levels are barely detectable in the middle-aged brain (Dame et al., 1999). However, in the present study, GM-CSF levels increased within the brainstem in response to prolonged (56 days) LPS exposure, but only in the middle-aged rats. GM-CSF is a pro-inflammatory factor released by astrocytes in response to IL-1β for LPS that binds to a specific receptor on microglia leading to up-regulation of MHC II expression (Pierson et al., 2012) and TLR4 gene expression (Parajuli et al., 2012) similar to that seen in the present study. GM-CSF can also enhance the LPS-induced NF-κB nuclear translocation and production of IL-1β (Parajuli et al., 2012), also similar to that seen in the present study. TNFα gene expression was increased by the LPS infusion but this increase was not influenced by the age of the animal. Typically, with normal aging, (XcT). The duration of the LPS infusion was responsible with a significant (p < 0.001) increase in gene expression of glutamate transporter 1 (GLT1) and TGFβ. In contrast, the duration of the LPS infusion was responsible with a significant decrease (p < 0.001) in the gene expression of tyrosine-hydroxylase (TH), TNFα, and brain-derived neurotrophic factor (BDNF) within the brainstem and dopamine-β-hydroxylase (DBH) gene expression within the hippocampus. The age of the rat was independently responsible for a significant (p < 0.001) decrease in the gene expression of BDNF and XcT. Each biomarker represents a minimum of 6 rats/group. Abbreviations: BDNF, brain-derived neurotrophic factor; DBH, dopamine-β-hydroxylase; GLT1, glutamate transporter 1; GM-CSF, granulocyte-macrophage-stimulating factor; IFNγ, interferon gamma; IL-1β, interleukin-1 beta; LPS, lipopolysaccharide; TGFβ, transforming growth factor-β; TH, tyrosine hydroxylase; TLR4, toll-like receptor 4; TNFα, tumor necrosis factor alpha; XcT, cysteine-glutamate anti-porter.
microglia release greater amounts of TNFα protein in response to LPS (Bardou et al., 2013; Colton and Wilcock, 2010; Harry, 2013); the discrepancy with our results may be because of age-related changes in posttranslational control of TNFα protein production (Chen et al., 2006). Finally, LPS also produced a duration-dependent increase in IL-13. IL-13 induces a class of protein-degrading enzymes, known as matrix metalloproteinases and may also contribute to the development of disorders that involve neuroinflammation such as amyotrophic lateral sclerosis (Shi et al., 2007), PD, and multiple sclerosis (Kim and Joh, 2012). Gene expression of the anti-inflammatory cytokine TGFβ was also significantly increased by the LPS infusion. TGFβ can, in turn, induce a persistent up-regulation of genes related to inflammation (Cacheaux et al., 2009). Higher concentrations of TGFβ have been found in the blood and cerebrospinal fluid of patients with PD or AD (Swartzfager et al., 2010).

CX3CR1 is neuroprotective and it is involved in terminating the expression of microglia pro-inflammatory biomarkers (Harry, 2013). Normal aging is typically associated with a down-regulation of the production of CX3CR1 proteins that may result from cell senescence (Wynne et al., 2010). In the present study, gene expression for this receptor was significantly increased following exposure to LPS; furthermore, this increase was independent of the age of the rat. This apparent disconnection between the influence of aging upon CX3CR1 gene expression and protein production may be because of senescence-induced changes in posttranslational control of protein production.

Dysregulation of glutamatergic neurotransmission may underlie the pathology associated with chronic neuroinflammation in the brain (Rosti et al., 2004; Wenk and Hauss-Wegrzyniak, 2001). TNFα can suppress GLT1 transcription (Sitcheran et al., 2005) and induce glutamate release from microglia (Takeuchi et al., 2006). IL-1β can trigger the release of glutamate from neurons (Liu et al., 2011) and inhibit GLT1 expression on astrocytes (Prow and Irani, 2008) and microglia (Takaki et al., 2012). Reactive oxygen species, such as...
those produced by activated microglia during neuroinflammation, inhibit glutamate uptake by astrocytes (Trotti et al., 1998). Increased extracellular glutamate also increases microglial release of proinflammatory signals (Taylor et al., 2005), which in turn would increase glutamate levels further leading to a positive feedback cycle. When extracellular glutamate levels are elevated, IL-1β can increase system XcT activity on astrocytes, resulting in increased neurotoxicity by glutamate (Jackman et al., 2010).

In the present study, LPS increased gene expression for IL-1β, TNFα, and GLT1, as well as IL-1β protein levels. As expected, XcT gene expression increased in parallel to the elevation in IL-1β gene and protein levels. Surprisingly, this compensatory increase in XcT gene expression was not observed in middle-aged and aged rats. XcT is a heteromeric Na⁺-independent anionic amino acid transport system that specifically facilitates the exchange of anionic amino acids for anionic forms of cystine and glutamate, thereby mediating the formation of glutathione within the neuron. Similarly, there was a duration-dependent increase in GLT1 gene expression in the young and middle-aged rats but not in the aged rats. We have previously shown by flow cytometry a significant increase in the number of CD11b/c-immunopositive microglia expressing GLT1 protein following chronic exposure to LPS (Brothers et al., 2013b). Taken together, our results suggest that with advanced age the brain’s ability to regulate glutamate levels and the production of glutathione are impaired, ultimately leading to increased neuronal vulnerability to the oxidative stress associated with chronic neuroinflammation.

LC degeneration occurs early in the progression of AD (Szot et al., 2006) and coincides with the presence of a pro-inflammatory environment (Cagnin et al., 2006). TH and DBH are precursors in the production of NE, the primary neurotransmitter found in LC projections to the hippocampus. We show a duration-dependent...
loss in TH gene expression in the midbrain and/or brainstem and reduced number of TH-IR cells in the LC and SNpc with LPS infusion and age. Overall, the oldest rats that experienced the longest duration of LPS infusion demonstrated the greatest decline in TH-IR cells in LC and SNpc, consistent with previous investigations (Mouton et al., 2012). The present study was designed to investigate for such an age-dependent increase in vulnerability to chronic neuroinflammation and identify a specific inflammatory biomarker underlying the LC cell loss in animal models of AD (Manaye et al., 2013). We anticipated that IL-6 might play a role in the age-related vulnerability (Ye and Johnson, 1999) but IL-6 levels in the present study only increased in response to the duration of the LPS infusion and was not dependent upon the age of the rat. BDNF signaling via trkB neurotrophin receptors is important for the maintenance of the LC innervation of the hippocampus (von Bohlen und Halbach and Minichiello, 2006); in the present study, aged rats infused with LPS expressed significantly less of the gene for BDNF than the younger rats infused with LPS.

Interestingly, after 56 days of LPS infusion, DBH is significantly increased in the hippocampus in all age groups compared with a decrease (although not significant) after 21 days of LPS infusion. This suggests a compensatory up-regulation in the DBH gene, consistent with our previous report (Brothers et al., 2013a). A decrease in TH enzyme levels in the midbrain and/or brainstem suggests a decline in catecholamine function that may underlie aspects of the cognitive impairment seen in AD (Grudzien et al., 2007). Cognitive decline may also be related to the fact that NE acts as an anti-inflammatory molecule within the cortex and hippocampus (Feinstein et al., 2002; Wenk et al., 2003), stimulates BDNF production (Mannari et al., 2008) and supports neurogenesis (Masuda et al., 2012); all functions that could be improved by a compensatory increase in hippocampal DBH. Overall, chronic neuroinflammation leads to impaired LC cellular integrity, reduced hippocampal neurogenesis (Marchalant et al., 2009), and reduced BDNF gene expression (Tong et al., 2008).

SNpc cellular degeneration is influenced by the presence of neuroinflammation early in the progression of PD (Tome et al., 2013; Wang et al., 2013). Previous inflammatory models of PD that injected LPS directly into the substantia nigra significantly reduced the number of TH-IR neurons (Herrera et al., 2000; Kim et al., 2000). In contrast, an acute intra-nigral injection of TNF-α (Castano et al., 2002) or an acute peripheral injection of a high dose of LPS both failed to reduce the number of TH SNpc neurons (Mouton et al., 2012). Similarly, we have demonstrated that a single acute injection of LPS into the nucleus basalis magnocellularis failed to reduce the number of acetylcholinergic neurons (Willard et al., 1999). In the present study, 3 weeks of continuous LPS infusion produced a decline in the number of TH-IR cells in young and middle-aged rats, but no additional decline in the number of TH-IR cells in the SNpc of aged rats. After 8 weeks of LPS infusion the number of TH-IR cells in the SNpc had completely recovered in young and middle-aged rats. In addition, the density of TH-IR cells in the SNpc was decreased because of normal aging; a decline in SNpc function that is consistent with previous reports (Gozlan et al., 1990; Miguez et al., 1999). Thus, the consequences of pro-inflammatory environment likely depend upon an interaction between the duration of the exposure to specific cytokines (Bardou et al., 2013), the age of the brain and the specific region. For example, the vulnerability of SNpc cells was primarily dependent upon the duration of the pro-inflammatory environment, while the vulnerability of LC cells was not changed by longer exposure to LPS. Overall, the infusion of LPS increased the density of MHC II-IR microglia and expression of pro-inflammatory cytokines (IL-1β and IL-1α) throughout the midbrain and/or brainstem.

Our data support the concept that continuous exposure to a pro-inflammatory environment drives exaggerated changes in the production and release of inflammatory mediators, decreased production protective factors, altered glutamate regulation and impaired cellular function within the SNpc, and LC. Furthermore, our data show that the response to a pro-inflammatory environment changes with age. Overall, these data suggest that early anti-inflammatory intervention is an important therapeutic opportunity in neurodegenerative diseases, which have defining pathology of neuroinflammation such as PD and AD.

Disclosure statement

The authors declare no conflicts of interest.

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