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Differential effects of duration and age on the consequences of neuroinflammation in the hippocampus

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ABSTRACT

The current study investigated the hypothesis that the duration of the proinflammatory environment plays a critical role in the brain's response that results in negative consequences on cognition, biochemistry, and pathology. Lipopolysaccharide or artificial cerebrospinal fluid was slowly (250 ηg/h) infused into the fourth ventricle of young (3-month-old), adult (9-month-old), or aged (23-month-old) male F-344 rats for 21 or 56 days. The rats were then tested in the water pool task and endogenous hippocampal levels of pro- and anti-inflammatory proteins and genes and indicators of glutamatergic function were determined. The duration of the lipopolysaccharide infusion, compared with the age of the rat, had the greatest effect on (1) spatial working memory; (2) the density and distribution of activated microglia within the hippocampus; and (3) the cytokine protein and gene expression profiles within the hippocampus. The duration- and age-dependent consequences of neuroinflammation might explain why human adults respond positively to anti-inflammatory therapies and aged humans do not.

1. Introduction

Advanced age and the duration of neuroinflammation might equally contribute to the frequent co-occurrence of Alzheimer's disease (AD) and Parkinson's disease (PD; Aarsland et al., 2001; Akiyama et al., 2000; Hobson and Meara, 2004; Hughes et al., 2000). Recent reports have shown that the co-occurrence of these disorders increases in a duration-dependent manner from 28% 6 years after diagnosis to approximately 83% 20 years after diagnosis (Hely et al., 2008; Perez et al., 2012). We and others have speculated that the consequences of neuroinflammation associated with microglial activation, operating across a time scale of decades, are carefully regulated until, because of normal aging, there is a gradual shift to a nonequilibrium state that is permissive for neurodegenerative processes (Colton and Wilcock, 2010; Smith et al., 2012; Wenk and Hauss-Wegrzyniak, 2001). Consistent with this hypothesis are epidemiological evidence that very long duration inflammatory diseases in humans, such as atherosclerosis, obesity, diabetes, depression, and periodontitis increase the risk of AD (Andersen et al., 2005; Balakrishnan et al., 2005; Biessels and Kappelle, 2005; Casserly and Topol, 2004; Dowlati et al., 2010; Kamer et al., 2009; Ownby et al., 2006).

Microglia activation is detectable many years before the onset of neuropathological changes associated with AD and PD and is predictive of later symptom severity, demonstrated by positron emission tomography studies (Cagnin et al., 2006; Gerhard et al., 2006; Imamura et al., 2003). Microglia can assume various activation states that are associated either with elevations of proinflammatory cytokines and the release of potentially destructive oxidative enzymes or the expression of a cytokine activation profile that sustains repair, recovery, and growth (Colton and Wilcock, 2010; Sudduth et al., 2013). This spectrum of activation states has been categorized as M1 or M2 in macrophages (Mantovani et al., 2004). Vulnerable brain regions, particularly the hippocampus, are likely exposed for many decades to a complex and varying blend of microglia in alternative activation states (Bilbo, 2010; Eikelenboom et al., 2010; Heneka et al., 2010; Herrup, 2010; Sudduth et al., 2013). The current study investigated the differential influence of brain age and the duration of the proinflammatory stimulus on the profile of hippocampal pro- and anti-inflammatory genes and proteins, the number of histologically identified activated microglia, and performance in a spatial memory task.

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2. Methods

2.1. Experimental design

Young, adult, and aged male F-344 rats received a chronic infusion of lipopolysaccharide (LPS), or its vehicle, into the fourth ventricle for 21 or 56 days and were then evaluated using behavioral, histological, biochemical, and genetic analyses. Multiple counter-balanced iterations of this study were performed so that at the end of the investigation each group contained 11 rats; thus, 132 rats completed all aspects of the investigation.

2.2. Subjects

Young (3-month-old), adult (9-month-old), and aged (23-month-old) male F-344 rats (Harlan Sprague-Dawley) were maintained on a 12/12-hour light/dark cycle in a temperature-controlled room (22 °C) with free access to food and water and with lights off at 9:00 PM. 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 given health checks, handled on arrival, and allowed at least 1 week to adapt to their new environment before surgery. We certify that the experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23), revised in 1996. We also certify that the formal approval to conduct the experiments was obtained from the animal subjects review board of Ohio State University.

2.3. Surgery

Artificial cerebrospinal fluid (aCSF; 140 mM NaCl, 3.0 mM KCl, 2.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, and 1.2 mM Na_2HPO_4 adjusted to pH 7.4) or LPS (0.25 μg/h, 1.66 mg/mL prepared in aCSF; *E. coli*, serotype 055:B5, TCA extraction; Sigma) were chronically infused via a cannula that was implanted into the fourth ventricle (−2.5 mm AP and −7.0 mm DV 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 Corp, Cupertino, CA, USA) as previously described (Hauss-Wegrzyniak et al., 1998; Marchalant et al., 2007; Rosi et al., 2005). Calculations using the average fill volume for this pump allows for release of LPS or aCSF for up to 56 days. Post-operative care included lidocaine 1% solution applied to the exposed skin on closure and 2 mL of isotonic saline via subcutaneous injection to prevent dehydration during recovery.

2.4. Behavioral testing: water pool task

The rats were handled daily for 5 days before behavioral testing began. Spatial learning ability was assessed for all rats using a 170-cm diameter water pool with gray walls. The water was maintained at room temperature (RT; 21 °C–22 °C). The pool was in the center of a room with multiple visual stimuli as distal and proximal cues. The circular escape platform was 10 cm in diameter. For the spatial (hidden platform) version of the water task, a circular escape platform was present in a constant location, submerged 2.5 cm below the water surface. The rats were tracked using Noldus Ethovision 3.1 tracking and analysis system (Noldus, Leesburg, VA, USA).

Each rat performed 6 trials per day for 4 consecutive days (24 trials total), with a 60-minute intertrial interval. The rat was released into the water on each trial from 1 of 7 locations spaced evenly at the side of the pool, which varied so that the rats did not start from any location twice in 1 day. After the rat found the escape platform or swam for a maximum of 60 seconds, it was allowed to remain on the platform for 30 seconds. At the end of the fourth day, the platform was removed and a standard probe trial was conducted. After the probe test, all rats were also tested with the platform raised 2 cm above the surface of the water to control for possible aging or LPS-induced deficits in visual acuity. The effects of aging and/or inflammation were assessed by the comparison of the latency to find the platform.

2.5. Histology

2.5.1. Tissue collection

All the rats were deeply anesthetized before sacrifice. Rats used for histology (5 rats per group) were prepared for a transcardiac perfusion with cold saline containing 1 U/mL heparin, followed by 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The brains were then postfixed overnight in the same fixative and then stored (4 °C) in phosphate-buffered saline (PBS), pH 7.4. Rats used for biochemistry (6 rats per group) from each group were briefly anesthetized and then rapidly decapitated; their hippocampi were quickly dissected on ice. The left and right hippocampi were randomly chosen for either protein or gene expression analyses. The hippocampi were stored at −80 °C until processed. Blood was collected during the rapid decapitation procedure. After centrifugation at 4 °C for 15 minutes at 2500*g*, serum was collected and assayed.

2.5.2. Immunocytochemistry

Free-floating coronal sections (40 μm) were obtained using a vibratome from perfused tissues for staining with standard avidin/biotin peroxidase labeling methods. The monoclonal antibody OX-6 (final dilution 1:200; Pharmigen, San Diego, CA, USA) was used to visualize activated microglial cells only. This antibody is directed against the class II major histocompatibility complex (MHC II) antigen, an indicator of activation (Akiyama et al., 2000; Streit and Xue, 2009). After quenching endogenous peroxidase activity and blocking nonspecific binding, the sections were incubated (4 °C) overnight with the primary antibody. Thereafter, the sections were incubated for 1.5 hours at RT with the secondary polyclonal antibody, rat adsorbed biotinylated horse anti-mouse immunoglobulin G (final dilution 1:200; Vector, Burlingame, CA, USA). Sections were then incubated for 1 hour at RT with avidin-biotinylated horseradish peroxidase (Vectastain, ABC kit; Vector). After washing again in PBS, the sections were incubated with 0.05% 3, 3'-diaminobenzidine tetrahydrochloride (Vector) as a chromogen. The reaction was stopped by washing the section with PBS. No staining was detected in the absence of the primary or secondary antibodies. Sections were mounted on slides, air-dried, and coverslipped with cytoseal (Allan Scientific, Kalamazoo, MI, USA) mounting medium. The location of immunohistochemically-defined cells was examined using light microscopy. Quantification of cell density in the reconstructed hippocampal coronal sections (at least 5 sections from each rat) was assessed with a Nikon 80i documentation system with a DS-5M-L1 digital camera using Elements 3.1 software (Nikon Instruments, Melville, NY, USA) and quantified using MetaMorph imaging software (Universal Image Corporation, West Chester, PA, USA).

2.6. Biochemistry

2.6.1. Protein analysis

Frozen (−80 °C) hippocampi were placed in a BioPlex Cell Lysis solution (Bio-Rad, Richmond, CA, USA) and total proteins were extracted according to the manufacturer's instructions. Hippocampal levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, IL-18 (interferon-γ