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A survey of neuroimmune changes in pregnant and postpartum female rats

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ABSTRACT

During pregnancy and the postpartum period, the adult female brain is remarkably plastic exhibiting modifications of neurons, astrocytes and oligodendrocytes. However, little is known about how microglia, the brain's innate immune cells, are altered during this time. In the current studies, microglial density, number and morphological phenotype were analyzed within multiple regions of the maternal brain that are known to show neural plasticity during the peripartum period and/or regulate peripartum behavioral changes. Our results show a significant reduction in microglial density during late pregnancy and the early-mid postpartum period in the basolateral amygdala, medial prefrontal cortex, nucleus accumbens shell and dorsal hippocampus. In addition, microglia numbers were reduced postpartum in all four brain regions, and these reductions occurred primarily in microglia with a thin, ramified morphology. Across the various measures, microglia in the motor cortex were unaffected by reproductive status. The peripartum decrease in microglia may be a consequence of reduced proliferation as there were fewer numbers of proliferating microglia, and no changes in apoptotic microglia, in the postpartum hippocampus. Finally, hippocampal concentrations of the cytokines interleukin (IL)-6 and IL-10 were increased postpartum. Together, these data point to a shift in the maternal neuroimmune environment during the peripartum period that could contribute to neural and behavioral plasticity occurring during the transition to motherhood.

1. Introduction

Becoming a mother is a transformational event in a female's life. Major hormonal shifts during pregnancy, parturition and the postpartum period coupled with experiential factors modify the brain and, as a result, the behavior of the female producing a high level of maternal responsiveness along with changes in maternal motivation, mood, cognition and stress regulation (Numan and Insel, 2003; Kinsley and Lambert, 2008; Leuner et al., 2010; Macbeth and Luine, 2010; Workman et al., 2012; Galea et al., 2014; Bridges, 2015; Agrati and Lonstein, 2016; Leuner and Sabihi, 2016). Peripartum neural modifications are numerous and occur within a distributed network of brain regions that regulate functions which are altered peripartum. Specifically, motherhood is accompanied by alterations in neurogenesis (Darnaudéry et al., 2007; Leuner et al., 2007; Pawluski and Galea, 2007), neuronal morphology (Rasia-Filho et al., 2004; Pawluski and Galea, 2006; Kinsley and Lambert, 2008; Leuner and Gould, 2010; Haim et al., 2014) and synaptic plasticity (Tomizawa et al., 2003) within areas such as the hippocampus (HPC), prefrontal cortex (PFC), basolateral amygdala (BLA), nucleus accumbens (NAc) and hypothalamus.

Plasticity in the maternal brain is not limited to neurons, as modifications of astrocytes (Featherstone et al., 2000; Salmaso et al., 2005; Salmaso and Woodside, 2006) and oligodendrocytes (Gregg et al., 2007; Maheu et al., 2009) have also been documented during pregnancy and/or the postpartum period. However, very little is known about possible peripartum-related changes in microglia, the brain's resident immune cells (Kettenmann et al., 2011), despite the fact that the maternal periperhal immune system is known to undergo dramatic changes during this time (Luppi, 2003; Aagaard-Tillery et al., 2006; Christiaens et al., 2008; Mor and Cardenas, 2010; Groer et al., 2015).

Microglia secrete cytokines and growth factors that are important regulators of neural plasticity under basal conditions. In the

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healthy brain, microglia regulate cell genesis, apoptosis, synaptogenesis, synaptic pruning and neural physiology (Schafer et al., 2012; Benarroch, 2013; Shigemoto-Mogami et al., 2014; Sierra et al., 2014; Walker et al., 2014; Lenz and McCarthy, 2015; Michell-Robinson et al., 2015; Patterson, 2015; Sato, 2015; Hong et al., 2016). Microglia are also highly responsive to hormones (Tanaka et al., 1997; Vegeto et al., 2001; Sierra et al., 2008; Karelina et al., 2011; Habib and Beyer, 2015; Yuan et al., 2016) and thus the dramatic hormonal changes occurring during the peripartum period (Numan and Insel, 2003) may alter microglial properties and function within the brain. As such, microglia are situated to be modulated by the unique hormonal milieu of motherhood and to in turn mediate neural plasticity known to occur in the brains of pregnant and postpartum females. Given the links between neuroimmune dysregulation and mood disorders such as depression and anxiety (Hodes et al., 2015; Yirmiya et al., 2015; Wohleb et al., 2016), there is also the underexplored possibility that microglial alterations may be relevant to understanding the pathophysiology of these conditions which are common during the peripartum period (Wisner et al., 2013).

Only one previous study has examined neuroimmune changes in mothers and it reported alterations in microglial density in the dorsal hippocampus (dHPC) on the day of parturition along with alterations in several immune molecules (Posillico and Schwarz, 2016). In the present study, we show that microglial density was reduced during late pregnancy and the early-mid postpartum period in the BLA, medial prefrontal cortex (mPFC), NAc and dHPC but not the motor cortex (mCTX). Further, microglia numbers were diminished postpartum in the four regions which showed a reduction in microglial density and this reduction occurred primarily in microglia with a thin, ramified morphology. The postpartum HPC also exhibited reduced numbers of proliferating microglia, but no change in apoptotic microglia, as well as higher levels of the cytokines interleukin (IL)-6 and IL-10. Together, these data show that the neuroimmune environment undergoes significant modifications during the peripartum period and in doing so broaden our understanding of the maternal brain with implications for also understanding potential neuroimmune contributors to peripartum-related plasticity and behavior.

2. Material and methods

2.1. Animals

Virgin female (200–250 g) and male (300–350 g) Sprague Dawley rats (Taconic, Albany, NY) arrived at our facility and were group housed for 1 week of acclimation. Colony conditions were maintained at a temperature of 23 ± 1 °C and relative humidity of 40–60% with a 12: 12 light:dark cycle (lights on at 6 AM). All rats had access to food and water *ad libitum*. All experiments were performed in compliance with The Ohio State University Institutional Animal Care and Use Committee, the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals and followed the ARRIVE guidelines.

For breeding, one male was placed with two virgin females in their home cage. Pregnancies were verified through daily vaginal swabs and microscopic identification of sperm. Upon positive determination of pregnancy, designated as gestation day 0 (GD0), female rats were individually housed. For the GD20 group, pregnancy was verified at the time of sacrifice by confirming the presence of embryos. For postpartum groups, the day of birth was designated as postpartum day (PD) 0, and on PD1, litters were culled to 8–10 pups (4–5 males, 4–5 females). Groups of virgin rats that weren't mated were individually housed after acclimation and their estrous cycles monitored via daily vaginal swabs. All animals were weighed daily.

2.2. Brain collection for immunohistochemistry

Separate groups of virgin rats (n = 11) in diestrus 1 (D1), pregnant rats (n = 6) on gestation day (GD) 20, and mother rats on PD1 (n = 6), PD8 (n = 12) or PD21 (n = 6) were deeply anesthetized with Euthasol (150 mg/kg; Virbac, St. Louis, MO) and transcardially perfused with 0.1 M phosphate buffered saline (PBS) followed by phosphate-buffered 4% paraformaldehyde. Brains were rapidly removed, post-fixed in 4% paraformaldehyde overnight at 4 °C and then transferred to 0.1 M PBS. A 1:12 series of 40 μm coronal sections were obtained using a VT1000S Leica Vibratome and stored in 0.1 M PBS at 4 °C until immunohistochemical staining.

2.3. Immunohistochemistry

Immunohistochemistry for Iba1, a constitutively expressed calcium-binding protein which is specific to microglia (Imai and Kohsaka, 2002; Ahmed et al., 2007), was performed as described elsewhere with slight modifications (Lenz et al., 2013). Briefly, sections were mounted onto slides and air dried for 1 h after which slides were rinsed in 0.1 M PBS 3 × 5 min and blocked with 10% bovine albumin serum (BSA) in 0.1 M PBS + 0.4% Triton-X for 1 h. Next, endogenous peroxidase activity was removed by a 1 h incubation in 0.3% H_2O_2 in 50% methanol. Slides were then rinsed with 0.1 M PBS 3 × 5 min and incubated with rabbit anti-Iba1 primary antibody (cat. # 019-19741; 1:1000; Wako, Richmond, VA) in 0.1 M PBS + 0.4% Triton-X + 5% BSA overnight at 4 °C. The specificity of this antibody has been verified by the manufacturer and by Horvath and DeLeo (2009) using western blot. The next day, slides were rinsed in 0.1 M PBS 3 × 20 min and incubated for 1 h at room temperature (RT) with biotinylated anti-rabbit secondary antibody (1:500; Vector Laboratories, Burlingame, CA) in 0.1 M PBS + 0.4% Triton-X + 2.5% NGS, followed by 1 h in ABC complex (1:500 each; Vector Laboratories) in 0.1 M PBS + 0.4% Triton-X. A 10 min incubation with Ni-DAB chromogen visualized the reaction product (2.5% Ni, 0.05% 3,3 diaminobenzidine tetrachloride, 0.005% H_2O_2 in 0.175 M sodium acetate). Slides were then thoroughly rinsed in PBS, dehydrated with ascending alcohol, cleared with xylenes and coverslipped with Permount.

Immunofluorescent staining was performed in order to co-localize: 1) Iba1 with the cell proliferation marker, Ki67 and 2) CD11b, a marker of macrophages and microglia, with the cell death marker, Caspase3. Briefly, sections were mounted onto slides, air dried for 1 h and rinsed with 0.1 M PBS 3 × 5 min. Sections were then blocked in 0.1 M PBS + 0.4% Triton-X + 5% BSA for 1 h at RT and rinsed again in 0.1 M PBS 3 × 5 min. For co-localization of CD11b and Caspase3, sections were incubated with primary mouse anti-CD11b (cat. # 550299; 1:100; BD Pharmingen, San Jose, CA) and a primary rabbit anti-Caspase3 (cat. # ab2302; 1:100; Abcam, Cambridge, MA) overnight at 4 °C. For co-localization of Iba1 and Ki67, sections were incubated with mouse/rat anti-Ki67 eFluor 570 (cat. # 41-5698-82; 1:100; eBiosciences, San Diego, CA) and a rabbit anti-Iba1 (cat. # 019-19741; 1:1000; Wako, Richmond, VA) in blocking solution overnight at 4 °C. Next, sections were rinsed in 0.1 M PBS 3 × 5 min and incubated with anti-rabbit (AlexaFluor488 cat. # A11070; 1:200; Life Technologies, Carlsbad, CA) and/or anti-mouse (DyLight549 cat. # DI-2549; 1:200; Vector Laboratories) secondary antibodies for 2 h at RT. Sections were then rinsed 3 × 5 in 0.1 M PB, coverslipped with DABCO and kept in the dark at 4 °C until imaging. Negative control sections, in which one primary antibody was omitted at a time, were performed for all experiments.