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Research report

Intrauterine inflammation reduces postnatal neurogenesis in the hippocampal subgranular zone and leads to accumulation of hilar ectopic granule cells

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ABSTRACT

Prenatal inflammation is associated with poor neurobehavioral outcomes in exposed offspring. A common route of exposure for the fetus is intrauterine infection, which is often associated with preterm birth. Hippocampal development may be particularly vulnerable to an inflammatory insult during pregnancy as this region remains highly neurogenic both prenatally and postnatally. These studies sought to determine if intrauterine inflammation specifically altered hippocampal neurogenesis and migration of newly produced granule neurons during the early postnatal period. Microglial and astroglial cell populations known to play a role in the regulation of postnatal neurogenesis were also examined. We show that intrauterine inflammation significantly reduced hippocampal neurogenesis between postnatal days 7 (P7) and P14 as well as decreased granule cell density at P28. Ectopic migration of granule cells was observed in LPS-exposed mice at P14, but not at P28. Intrauterine inflammation had no effect on hippocampal astrocyte or microglia density or on apoptosis rate at the postnatal time points examined. Thus, exposure to intrauterine inflammation disrupts early postnatal neurogenesis and leads to aberrant migration of newly born granule cells.

1. Introduction

Exposure to prenatal inflammation is associated with adverse long-term neurobehavioral outcomes for exposed infants, including learning and sensory-motor deficits, epilepsy, schizophrenia and autism spectrum disorder Allen, 2008; Brown et al., 2009; Ellman et al., 2009; Fatemi et al., 2002; Hultman et al., 1999; Knuesel et al., 2014; Mednick et al., 1988; Meyer, 2013. One of the most common clinical scenarios by which a fetus is exposed to inflammation is in the setting of preterm birth which is associated with local infection or inflammation in the uterus. It has been suggested that intrauterine inflammation disrupts a critical window of brain development, leading to an array of postnatal deficits Dammann and Leviton, 1997. Animal studies support a role for altered glial development ("white matter damage") as well as neuronal injury as a proposed mechanism by which exposure to intrauterine inflammation leads to adverse neurological outcomes Boksa, 2010; Burd et al., 2012; Hagberg et al., 2015. However, the effect of prenatal inflammation on distinct brain areas with well characterized behavioral functions still remains largely unknown.

The role of the hippocampus in learning and memory has been well characterized Eichenbaum et al., 2012; Gaesser et al., 2013; Schacter et al., 2007 and is thought to rely on continuing neuronal production which takes place in the hippocampal subgranular zone (SGZ) of the postnatal brain Aimone et al., 2014; Lee et al., 2013; Ming and Song, 2011; Mu and Gage, 2011. The SGZ supports neural stem and progenitor cells (NPCs) which undergo repeated mitotic divisions Kempermann et al., 2015 producing differentiating cellular progeny which, upon completion of differentiation and maturation stages, become fully functional dentate granule neurons that are indiscernible morphologically and physiologically from granule cells born *in utero* Ambrogini et al., 2004; Ramirez-Amaya et al., 2006; Toni et al., 2008; van Praag et al., 2002. In the process of maturation, the majority of newborn cells navigate radially within the granule cell layer (GCL); however, some of these cells fail to retain their normal position and migrate further into the hilar area Hester and Danzer, 2014. These so called hilar ectopic granule cells (hEGCs) develop abnormal synaptic connectivity and show altered morphological and electrophysiological properties compared to the granule cells that reside within the GCL Scharfman and Pierce, 2012; Scharfman et al., 2007; Zhan et al., 2010. An unusually high

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presence of hEGCs has been documented in animal models of epilepsy and is thought to contribute to the development of this disorder Cayre et al., 2009; Hester and Danzer, 2014.

Hippocampal neurogenesis is a highly dynamic process known to be regulated by various environmental, physiological and pathological stimuli Aimone et al., 2014. In particular, it has been shown that systemic maternal inflammation during pregnancy can negatively affect postnatal neurogenesis in the hippocampal SGZ Green and Nolan, 2014. However, the role of intrauterine inflammation, as might occur in spontaneous preterm birth, on hippocampal neurogenesis has not been investigated in much detail Jiang et al., 2012. We hypothesize that exposure to intrauterine inflammation during a critical period of brain development will specifically alter hippocampal neurogenesis in the postnatal brain. In addition, microglial and astroglial cell populations have been shown to play a role in modulating neuronal production in the adult brain. In particular, microglia, which represent resident brain macrophages Kreutzberg, 1996; Town et al., 2005, are responsible for reducing the rate of postnatal neurogenesis following both systemic Monje et al., 2003 and local (within the brain) Ekdahl et al., 2003 inflammatory stimuli. Similarly, astrocyte function is also involved in the regulation of hippocampal neurogenesis under both normal Ashton et al., 2012; Környei et al., 2007 and inflammatory conditions Vallieres et al., 2002. Using our previously established model of inflammation-induced fetal brain injury Burd et al., 2010a,b; Elovitz et al., 2006, this study sought to address the effect of *in utero* inflammation on early postnatal neurogenesis and granule cell migration in the hippocampal SGZ as well as to examine possible alterations in microglial and astroglial cell populations.

2. Results

2.1. Exposure to intrauterine inflammation reduces early postnatal neurogenesis

Alterations in hippocampal neurogenesis were determined by labeling mitotically active NPCs with BrdU on postnatal days 7 and 8 and quantifying their neuronal progeny a week later, on P14 (Fig. 1A and B). Dual labeling with BrdU and the neuron-specific marker NeuN identified newly generated granule cells within the upper and lower blades of the dentate cell body layer (Fig. 1B). A significant reduction in the density of BrdU-labeled cells was detected in pups exposed to intrauterine inflammation as compared to control saline-injected animals (Fig. 1C, $p = 0.023$).

2.2. Intrauterine inflammation leads to decreased neuronal density in the dentate gyrus at P28

Since we observed inflammation-induced decline in hippocampal neurogenesis, granule neuron density and GCL thickness were assessed at P14 and P28 using a neuronal marker Prox1. While granule cell density was not different between control and experimental groups at P14, it was reduced at P28 after exposure to inflammation (Fig. 2A, $p = 0.002$). GCL thickness was not affected at either time point (Fig. 2B).

2.3. Exposure to intrauterine inflammation generates ectopic granule cells

Prox1 labeled granule cells in the hilus of inflammation-exposed pups and saline-exposed pups were compared to ascertain if any granule cells were migrating improperly (Fig. 2C). Following exposure to intrauterine inflammation, the number of hEGCs was significantly increased at P14 ($p = 0.023$) (Fig. 2C and D). This effect was not apparent at P28 (Fig. 2C).

2.4. Intrauterine inflammation has no effect on total number and division rate of neural stem and progenitor cells at P14 and P28

The total number of hippocampal stem and progenitor cells was measured by counting nestin-positive cells in the SGZ of inflammation-exposed and control animals (Fig. 3A). The density of NPCs was not altered between exposed and unexposed pups at either P14 or P28 (Fig. 3B). Additionally, exposure to prenatal inflammation did not affect the number of mitotically active cells within the SGZ (Fig. 3C and D). Hence, there were no differences in Ki67-positive cell densities present at either time point.

2.5. Intrauterine inflammation has no effect on postnatal apoptosis in the dentate gyrus

Apoptotic cells measured by labeling with an antibody against Cleaved Caspase 3 (Fig. 4A) were sparse in both treatment groups and their presence did not change in response to intrauterine inflammation at either time point (Fig. 4B).

2.6. Intrauterine inflammation has no effect on hippocampal microglia and astroglia densities

Microglial density was quantified in various regions of the hippocampus, including hilus, dentate gyrus (DG) and Cornu ammonis 1-3 (CA1-3), using antibodies against microglial marker Iba1 (Fig. 5A). Our data show that the number of microglial cells did not vary by exposure at P14 (Fig. 5B) or P28 (not shown).

The astrocyte specific marker Glial Fibrillary Acidic Protein (GFAP) was used for measuring astroglial density in the hippocampus (Fig. 6A). Similar to findings with microglia, exposure to intrauterine inflammation did not alter the number of astrocytes in the various regions of the hippocampus in any of the regions analyzed at P14 (Fig. 6B) or P28 (not shown).

3. Discussion

Exposure to prenatal inflammation has been strongly associated with a spectrum of adverse neurobehavioral outcomes in exposed offspring, including motor-sensory deficits, delayed learning and neurological disease, such as schizophrenia, autism spectrum disorder and epilepsy Anderson and Doyle, 2003; Cordeiro et al., 2015; Fazzi et al., 2009; Hack et al., 2005; Indredavik, 2010; Johnson et al., 2010a, b; Lee et al., 2011; Schieve et al., 2010. However, there is still a paucity of data on how an inflammatory environment *in utero* leads to fetal brain injury, causing neurobehavioral abnormalities in childhood and adulthood. In this study, we demonstrate that in addition to previously reported white matter damage and neuronal injury Burd et al., 2010a,b; Elovitz et al., 2006; Nitsos et al., 2006, early exposure to intrauterine inflammation results in a reduced rate of neurogenesis in the hippocampal SGZ and the accumulation of ectopic dentate granule cells known to be implicated in synaptic malfunction and increased neuronal excitability. As altered hippocampal function is associated with many neurological deficits, these findings provide a new mechanism by which exposure to intrauterine inflammation may lead to long term adverse outcomes.

The finding of decreased neuronal production in the hippocampal SGZ in response to intrauterine inflammation is consistent with a number of previous studies made in rodent model systems which reveal a reduction in postnatal neurogenesis following systemic inflammatory insults, including those evoked by *E. coli* LPS Cui et al., 2009; Girard et al., 2012; Graciarena et al., 2010, 2013; Lin and Wang, 2014; Mouihate, 2016. In contrast, a local *E. coli* administration in pregnant rats, which was shown to induce an