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NEONATAL BINGE ALCOHOL EXPOSURE INCREASES MICROGLIAL ACTIVATION IN THE DEVELOPING RAT HIPPOCAMPUS

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Abstract—Aberrant activation of the developing immune system can have long-term negative consequences on cognition and behavior. Teratogens, such as alcohol, activate microglia, the brain's resident immune cells, which could contribute to the lifelong deficits in learning and memory observed in humans with Fetal Alcohol Spectrum Disorders (FASD) and in rodent models of FASD. The current study investigates the microglial response of the brain 24 h following neonatal alcohol exposure (postnatal days (PDs) 4–9, 5.25 g/kg/day). On PD10, microglial cell counts and area of cell territory were assessed using unbiased stereology in the hippocampal subfields CA1, CA3 and dentate gyrus (DG), and hippocampal expression of pro- and anti-inflammatory genes was analyzed. A significant decrease in microglial cell counts in CA1 and DG was found in alcohol-exposed and sham-intubated (SI) animals compared to undisturbed suckle controls (SCs), suggesting overlapping effects of alcohol exposure and intubation alone on the neuroimmune response. Cell territory was decreased in alcohol-exposed animals in CA1, CA3, and DG compared to controls, suggesting the microglia have shifted to a more activated state following alcohol treatment. Furthermore, both alcohol-exposed and SI animals had increased levels of pro-inflammatory cytokines IL-1β, TNF-α, CD11b, and CCL4; in addition, CCL4 was significantly increased in alcohol-exposed animals compared to SI as well. Alcohol-exposed animals also showed increased levels of anti-inflammatory cytokine TGF-β compared to both SI and SCs. In summary, the number and activation of microglia in the neonatal hippocampus are both affected in a rat model of FASD, along with increased gene expression of pro- and anti-inflammatory cytokines. This study shows that alcohol exposure during development induces a neuroimmune response, potentially contributing to long-term alcohol-related changes to cognition, behavior and immune function.

Key words: FASD, neuroimmune, development, cytokines, pro-inflammatory.

INTRODUCTION

Prenatal alcohol exposure can lead to the development of serious cognitive and behavioral deficits which arise from structural and functional changes in the brain. Fetal Alcohol Spectrum Disorders (FASD) are estimated to affect up to 5% of live births each year (Sampson et al., 1997; May et al., 2009; CDC, 2015), making prevention and treatment of these disorders of the utmost importance. Brain regions such as the hippocampus and prefrontal cortex are vulnerable to the teratogenic effects of alcohol during the "brain growth spurt" which occurs during the third trimester of pregnancy in humans and the first two postnatal weeks in rats (Dobbing and Sands, 1979; Mooney et al., 1996; Goodlett and Eilers, 1997; Klintsova et al., 2007), making alcohol exposure during this time window particularly devastating for these brain areas.

Recent work has suggested alcohol-induced neuroinflammation, as measured by increased activation of microglia and levels of associated cytokines, as a potential secondary source of damage in various models of alcohol exposure, both during development and in adulthood (Saito et al., 2010; Kane et al., 2011; McClain et al., 2011; Tiwari and Chopra, 2011; Marshall et al., 2013; Drew et al., 2015; Topper et al., 2015). Alcohol exposure during the third trimester-equivalent induces waves of apoptosis in the hippocampus, possibly triggering microglial migration to the damaged tissue and activation of the resident microglia to phagocytose dying cells and debris (Miller, 1988; Ikonomidou et al., 2000; Smith et al., 2015). Microglial activation in response to alcohol has been suggested to not only be a consequence of the insult also but a source of inflammation and tissue damage (Marshall et al., 2013).

The rodent immune system begins development during gestation and continues through the first two weeks of neonatal life, with microglia beginning colonization on embryonic days 9–10 in a brain-region-specific manner (Chan et al., 2007; Ginhoux et al., 2010). Microglia respond to immune challenges through release of pro- and anti-inflammatory cytokines and phagocytosis of dying neurons and pathogens. Aberrant microglia activation during development can lead to chronically increased levels of pro-inflammatory cytokines

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Abbreviations: AE, alcohol-exposed; ANOVA, analysis of variance; BACs, blood alcohol concentrations; DG, dentate gyrus; FASD, Fetal Alcohol Spectrum Disorders; Iba-1, ionized calcium-binding adaptor molecule 1; LTP, long-term potentiation; PD, postnatal day; SC, suckle control; SEM, standard error of the mean; SI, sham-intubated; TBS, Tris-buffered saline.

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which could lead to neurodevelopmental and psychopathological disorders (Cai et al., 2000; Urakubo et al., 2001; Meyer et al., 2006) or exaggerated immune responses to challenges later in life (Bilbo and Schwarz, 2009, 2012).

Microglia can express both pro- and anti-inflammatory cytokines in response to an immune challenge. The release of cytokine and phagocytosis of debris is linked with microglial morphology (Gehrmann et al., 1995; Neumann et al., 2009). Microglia exhibit various activation states characterized by changes to their physical shape and size. Resting or quiescent microglia are characterized by a small soma and long, thin processes for surveying the local microenvironment for pathogens or injury (Fig. 1A). Once a pathogen has been detected the microglia's soma enlarges and its processes shorten and thicken. A fully activated microglia displays a round, amoeboid shape with either very short or complete lack of processes (Fig. 1B; Nimmerjahn et al., 2005; Olah et al., 2011; Fu et al., 2014). Pro-inflammatory cytokines, released by activated microglia and macrophages, have cytotoxic effects and can induce further cell loss and tissue damage, while anti-inflammatory cytokines inhibit expression of pro-inflammatory cytokines, initiate cellular repair, and are generally thought to be neuroprotective. A balance between the actions of these cytokines dictates how the brain recovers from immune challenges.

The current study investigates whether third trimester-equivalent (postnatal days [PD]) 4–9) binge-like alcohol exposure affects microglial activation in the neonatal rat hippocampus through analysis of subregion-specific microglial number and territory (area) occupied by microglial body and processes. Cell territory provides an indirect measure of activation state, as a smaller area would indicate a more activated morphology (Drew and Kane, 2014). Neuroinflammation was also measured through analysis of gene expression of four pro-inflammatory cytokines: IL-1β, TNF-α, CD11b, and CCL4, and one anti-inflammatory cytokine: TGF-β. We hypothesized that our model of alcohol exposure would increase the number of microglial cells in the hippocampus of neonatal rats, activate existing microglia, and increase production of pro-inflammatory cytokines. This study will give a starting point for assessing whether aberrant neuroimmune activation via neonatal alcohol exposure could have long-term consequences for the brain and behavior.

EXPERIMENTAL PROCEDURES

Animals

Timed-pregnant Long-Evans rat dams were acquired from Harlan Laboratories (Indianapolis, IN, USA) and housed in cages of standard dimensions (17 cm

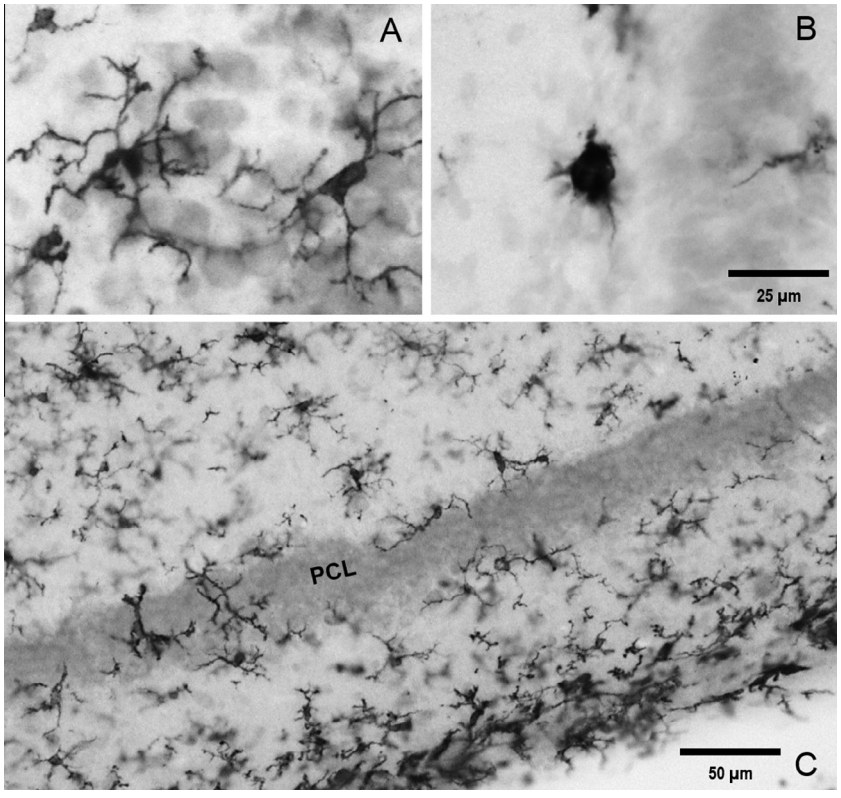

Fig. 1. Representative images of Iba-1+ microglia in the postnatal day 10 rat hippocampus. (A) Quiescent microglia with small somas and long, thin processes. (B) Fully activated amoeboid microglia with a large, round soma and very short processes. Both images taken with a 40× lens. (C) Iba-1 immunostaining in the neonatal rat hippocampal CA1. Image taken with a 20× lens.