Brain, Behavior, and Immunity 63 (2017) 88–98

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Special Issue on Perinatal Inflammation

# Prenatal infection leads to ASD-like behavior and altered synaptic pruning in the mouse offspring

Lourdes Fernández de Cossío [a], Andrea Guzmán [a], Suzanne van der Veldt [a,b], Giamal N. Luheshi [a,*]

[a] Douglas Mental Health University Institute, Department of Psychiatry, McGill University, Quebec, Canada
[b] Nutrition et Neurobiologie Intégrée, INRA UMR 1286, 33076 Bordeaux, France; University of Bordeaux, Bordeaux, France

ARTICLE INFO

*Article history:*
Received 27 July 2016
Received in revised form 23 September 2016
Accepted 29 September 2016
Available online 30 September 2016

*Keywords:*
LPS
Microglia
Maternal immune activation
ASD
Synaptic pruning
Fractalkine
Complement components

ABSTRACT

Environmental challenges to the maternal immune system during pregnancy have been associated with an increase in the frequency of neurodevelopmental disorders such as Autism Spectrum Disorders (ASD) appearing in the offspring. Microglia, the brain's resident immune-cells, are now known to be critically involved in normal brain development, shaping connections between neurons by pruning superfluous synaptic spines. Our aim was to investigate whether maternal infection during critical stages of gestation compromises the role of microglia in sculpting neuronal circuits. Using a mouse model of maternal immune activation (MIA) induced by bacterial Lipopolysaccharide (LPS), we assayed the offspring's behavior during postnatal development. Additionally, we quantified spines within the offspring's brain and assessed alterations in some molecular signals involved in pruning. LPS-induced MIA led to behavioral changes relevant to ASD in the offspring in the absence of gross neurological problems. Prenatal LPS resulted in a significant increase in the number of spines in the granule cells of the dentate gyrus, as well as a reduction in hippocampal expression of the fractalkine microglial receptor (CX3CR1), involved in mediating the pruning process in the offspring. Interestingly, these changes were only noted in the male progeny of the LPS challenged dams. These results provide an early indicator that microglial function is altered in the brain of offspring from immune challenged mothers and that the effects in the brain appear to be specific along sex lines.



## 1. Introduction

The highly complex process of brain development is under tight temporal and spatial regulation. Yet despite its various barriers, defenses, and redundancies, it is particularly sensitive to both endogenous and exogenous factors that may significantly alter the developmental trajectory of cells, neural circuits, and associated behavioral outcomes (Bilbo and Schwarz, 2012). A number of environmental (Estes and McAllister, 2015) and genetic (Oskvig et al., 2012; Sullivan et al., 2012) factors, as well as their interaction, have been suggested to disrupt neuronal structure and function, which can ultimately result in disorders like Schizophrenia, Attention-Deficit/Hyperactivity Disorder (ADHD) and Autism Spectrum Disorders (ASD). However, the resulting neurodevelopmental alterations may only become apparent postnatally as processes like myelination or synaptic pruning are prolonged in the higher-order neural structures functionally associated with these conditions (Paolicelli et al., 2011). In the case of ASD specifically, impairment in socio-emotional processing and repetitive, restricted behaviors, interests or activities are key features. This makes social interaction and communication especially difficult, thus making the management of this disorder a burdensome task. ASD is much more common in males than females, with a sex ratio of approximately four to one across the whole autism spectrum, yet sex-specific differences are rarely addressed in animal models (Bao and Swaab, 2010; Mandy et al., 2012).

Several environmental factors that have been associated with an increased risk of ASD include advanced maternal age, maternal obesity, gestational diabetes, maternal autoimmunity, and maternal infections (Estes and McAllister, 2015), with converging evidence from human and animal studies suggesting maternal immune activation (MIA) during pregnancy as the shared underlying characteristic of these conditions (Ashwood et al., 2011; Patterson, 2011). Thus, these various environmental factors may exert their effect on neurodevelopment through a common mechanism: evoking an immune response in the mother (Ellman and Susser, 2009).

Animal studies have echoed epidemiological findings, showing that MIA leads to neurodevelopmental alterations (Boksa, 2010;

* Corresponding author at: Douglas Mental Health University Institute, Department of Psychiatry, McGill University, 6875 LaSalle Boulevard, Montreal, Quebec H4H 1R3, Canada.
*E-mail address:* giamal.luheshi@mcgill.ca (G.N. Luheshi).

http://dx.doi.org/10.1016/j.bbi.2016.09.028
0889-1591/

Knuesel et al., 2014; Smith et al., 2007). The effects of MIA on the offspring's endocrine, immune, and nervous system have been widely studied in the last decade (Ashdown et al., 2006; Eyo and Dailey, 2013; Garay et al., 2013; Patterson, 2011). At the macroscopic level, studies have found abnormalities in both gray and white matter, in the neocortex and corpus callosum, with increased numbers of microglia in the forebrain white matter (Girard et al., 2010; Larouche et al., 2005; Paintlia et al., 2008). These observations relate to changes observed at the cellular level, where studies report oligodendrocyte damage (Graf et al., 2014), an increase in apoptosis (Kumral et al., 2007; Paintlia et al., 2008), and decreased neurogenesis in the hippocampus (Cui et al., 2009; Mattei et al., 2014). These alterations provide a striking parallel to the neuropathology found in studies on children with ASD (Sinha et al., 2015).

Both bacterial and viral infections during pregnancy have been modeled in rodents, using three main immunogenic approaches: Lipopolysaccharide (LPS), polyinosinic:polycytidylic acid (poly IC), or influenza virus. However, due to the wide variety of type, dose, and timing of immunogen across studies, comparison between findings is challenging. Few groups have studied behavioral effects of a single injection of LPS between E14 and E17 in mice specifically. Some of the existing findings include, decreased associative learning, increased novel object recognition, increased anxiety, and increased open field exploration in an LPS-induced MIA mouse model (Golan et al., 2005, 2006a,b). Additionally, decreased reversal learning of left-right discrimination, decreased working memory in water maze, and increased amphetamine- and MK801-induced locomotion in adulthood have also been reported (Meyer, 2006; Meyer et al., 2008), as well as increased anxiety and immobility in male adult offspring (Enayati et al., 2012). Here, we use an LPS-induced MIA mouse model and show behavioral alterations for males and females that fall within the two main diagnostic criteria of ASD: social/communicative behavior and repetitive/stereotypic movements or actions.

The precise mechanism by which prenatal inflammation causes altered neurodevelopment thus far also remains unclear. However, a number of recent studies have implicated microglia as being functionally important (Estes and McAllister, 2015; Malkova et al., 2012; Patterson, 2009, 2011; Takano, 2015). Given the role of microglia in both the onset and modulation of inflammation as well as brain development (Bilimoria and Stevens, 2015; Hong et al., 2016; Kettenmann et al., 2011), these cells could provide a mechanistic basis for the pathogenesis and of neurodevelopmental disorders and their sex differences (Schwarz and Bilbo, 2012; Salter and Beggs, 2014; Takano, 2015). We analyzed behavioral alterations relevant to ASD as an example of a well characterized neurodevelopmental disorder and a relevant one for investigating early changes in neurodevelopment. To explore potential alterations of microglial physiological function during network formation, we looked at a time point (P15) and a structure (hippocampus) where effects would be most readily detected for this neurobiological process as previously reported (Paolicelli et al., 2011). We show that a single prenatal inflammatory event affects offspring at both the behavioral and synaptic levels and we provide evidence that implicate microglia as possible mediators of these effects. Interestingly, discrepancy between behavioral and brain data are found in our analysis by sex lines.

## 2. Materials and methods

### 2.1. Animal breeding and maternal immune activation

All protocols were approved by McGill University's Animal Care Committee under the guidelines of the Canadian Council on Animal Care. Female C57/BL6 mice of reproductive age (between 6 and 8 weeks old; Charles River) were housed up to 4/cage and left to habituate to the new housing conditions for ten days under a 12 h light cycle (8 am–8 pm), with *ad libitum* access to food and water. Females were transferred to new cages (2/cage) and male bedding added two days prior to male presentation. Male mice were housed 1:2 females for one or two days until seminal plug appeared, which was considered embryonic day 0 (E0), and each female was then weighed and moved into a new cage with nesting material. Animals were weighed again at E7 and E15 to confirm pregnancy and to administer the injection respectively.

LPS, 100 μg/kg (E. coli serotype 0111:B4, Lot. 011M4008V, Sigma, Canada), a $Gm^-$ component of the bacterial cell wall was used in all studies as previously described by us (Ashdown et al., 2006), while saline vehicles were used for controls. LPS or saline injections were administered to dams ($N = 6–7$ per treatment condition) intraperitoneally at E15, when they were between 10 and 12 weeks old. This time point is in the second half of mouse gestation, which roughly corresponds to the late first or early second trimester in human gestation, when infections confer the most risk to ASD in the human offspring (Atladóttir et al., 2010; Boksa, 2010).

### 2.2. Behavior

ASD-like behaviors were explored in the offspring of LPS treated mothers using established protocols assessing: 1) early-onset of difficulties in social interaction and communication by measuring ultrasonic vocalizations at P8 as well as social interest in adulthood at 9 weeks of age using the three-chamber test and, 2) repetitive, restricted behaviors, interests and activities by measuring marble burying at 8 weeks of age as previously used (Angoa-Pérez et al., 2013; Kim et al., 2016; Malkova et al., 2012) and stereotypic behavior in the open field at 10 weeks of age. Behavior experiments were performed throughout development in the same offspring, with a maximum of two offspring of the same sex used per litter, blindly assigned to the behavioral protocol from 3 to 4 different dams per experimental condition. In addition, we evaluated righting and geotactic reflexes at P4 and P5 to assess early neurological well-being of all pups. At P24, mice were weaned into new cages separated by sex and placed onto a ventilated rack. The following subsections detail the modifications made to the behavior protocols followed.

#### 2.2.1. Righting and negative geotaxis reflex

The measured reflexes were based on previously developed reflexologic tests (Fox, 1965) and were conducted following existing methodology with some minor modifications (Baharnoori et al., 2012). In the negative geotactic reaction, the pup (age P4 and P5) was positioned face down on a sandpaper surface with a 35-degree inclination. The time taken until the pup positioned itself face up was measured with a cutoff at 120 s.

#### 2.2.2. Ultrasonic vocalization

Isolation-induced ultrasonic vocalizations were measured following published protocols (Hofer et al., 2001; Baharnoori et al., 2012). In the test room, a Mini-3 tunable bat detector was suspended 15 cm above the floor of a plexiglass box [20.3 (l) × 20.3 (w) × 20.3 (h) cm] where the pups (age P8) were placed. The frequency of the detector was set to 50 kHz and ultrasonic calls were recorded for 5 min. After each pup was tested, its weight was recorded before placing it back with the rest of the litter, and the plexiglass box was cleaned with 20% ethanol. Data was collected and analyzed using UltraVox software (Noldus Information Technology, Leesburg, VA).