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EARLY PRENATAL EXPOSURE TO LPS RESULTS IN ANXIETY- AND DEPRESSION-RELATED BEHAVIORS IN ADULTHOOD

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Abstract—Maternal immune activation can result in different behavioral abnormalities and brain dysfunction, depending on the nature of the inflammogen and the timing of the challenge. Few studies report the possible link between prenatal exposure to inflammation and mood disorders. Here we aimed to evaluate the effects of a single low lipopolysaccharide (LPS) injection to the dam at gestational day 9 on the offspring behavior and hippocampal function. We found that mice exposed to LPS show anxiety- and depression-related behaviors. Specifically, we found that animals prenatally exposed to LPS avoided the open arms of an elevated plus maze, the center of an open field and the lit side of a light/dark box, and they spent more time immobile in both the forced swimming and tail suspension tests, when compared with offspring of saline-injected dams. In addition, LPS mice had reduced serotonin and noradrenaline levels in the hippocampus and diminished Reelin immunoreactivity in the dentate gyrus, while their adult hippocampal neurogenesis was not affected. Results presented here support specific long-term effects of the response to a bacterial immunogen early in pregnancy, as opposed to different effects previously reported of viral immunogens and/or responses in late pregnancy. Our work adds to recent reports and stresses the relevance of considering prenatal exposure to a maternal immune response as a risk factor for mood disorders.
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Key words: maternal immune activation, Reelin, neurogenesis, serotonin, mood disorders.

INTRODUCTION

Prenatal infection has been associated with the development of various psychiatric disorders, including autism and schizophrenia (Meyer, 2013). A developing foetus can be exposed to the maternal immune response elicited by bacteria, viruses or parasites, as well as the result of allergy or autoimmune diseases. Even stress can affect the maternal immune state (Veru et al., 2014). Although maternal and perinatal medical care has improved survival rates of newborns from mothers suffering from infections or trauma, the maternal response can still affect the foetus inflammatory status and then alter normal neurodevelopment, leaving enduring alterations in brain function.

In the recent years, different animal models have been used in an effort to understand the different effects of prenatal infection on brain physiology as well as the underlying mechanisms (Meyer, 2013). In rodents, perinatal inflammatory stimuli can result in long-lasting behavioral and physiological alterations and the effects observed depend on the timing, dose and nature of the inflammogen used (Bilbo and Schwarz, 2012).

Due to a significant link between schizophrenia and maternal infection with influenza virus, several researchers have inoculated rodents perinatally with poly riboinosinic-polyribocytidilic acid (PolyI:C) (Meyer, 2013; Reisinger et al., 2015). PolyI:C is a synthetic analog of double-stranded RNA which activates toll-like receptor (TLR) 3, thus emulating a viral infection. Mice prenatally exposed to PolyI:C show long-term alterations in behavior and the inflammatory response (Meyer et al., 2006b, 2009). Nevertheless, the effects on the offspring depend on the developmental age at which they are exposed to the maternal inflammatory response. When inflammation is elicited late in pregnancy (GD15–17), offspring shows schizophrenia-related behavior (Meyer et al., 2006b; Zhang and van Praag, 2014), reduced hippocampal volume (Piontkewitz et al., 2011), alterations in neurotransmission (Ducharme et al., 2012), reduced neurogenesis and changes in the physiology of the new neurons (Zhang and van Praag, 2014). When offspring is exposed to the PolyI:C at GD9, however, they show reduced exploratory behavior (Meyer et al., 2006b), less Reelin-positive cells in the CA1 (Meyer et al., 2006b; Harvey and Boksa, 2012), and reduced neurogenesis (Meyer et al., 2006b).

Not only viral infections, but also bacterial infections are common during pregnancy. Actually, bacterial vaginosis has a very high prevalence in women of reproductive age

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Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; A, adrenaline; BrdU, 2-bromo-5-deoxyuridine; C57, C57BL/6J strain; DCX, doublecortin; DG, dentate gyrus; EDTA, ethylenediaminetetraacetic acid; GD, gestational day; HPLC-EC, high-pressure liquid chromatography and electrochemical detection; LPS, lipopolysaccharides; MANOVA, multiple analysis of variance; NA, noradrenaline; PD, postnatal day; PFA, paraformaldehyde; PolyI:C, Polyriboinosinic–polyribocytidilic acid; Sal, saline; SGZ, subgranular zone; TLR, toll-like receptor.

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(29%), although it is commonly asymptomatic and thus not treated (Koumans et al., 2007). Therefore, the study of long-term effects of the maternal inflammatory response to bacterial infection is relevant to understanding its contribution to adult brain function. Different animal models have been developed using lipopolysaccharides (LPS) to mimic bacterial infection. LPS is a component of the outer membrane of Gram-negative bacteria recognized by TLR 4 and consequently produces a strong inflammatory effect upon injection in mammals. Again, the effects observed in the offspring after eliciting an inflammatory response in the dam depend on the developmental age and the dosage. In rats, offspring exposed to LPS at GD10.5 showed increased anxiety- and depression-related behaviors and reduced serotonin levels in the hippocampus (Wang et al., 2009; Lin et al., 2012; Lin and Wang, 2014). In mice, prenatal LPS at GD17 can result in increased anxiety and fear in adulthood (Hava et al., 2006) and affect learning (Golan et al., 2005). A high dose of LPS (0.3 mg/kg) injected subcutaneously at GD8 results in abnormal behavior in the novel object recognition task (Coyle et al., 2009).

Here, we aimed to analyze the long-lasting effects of the maternal inflammatory response at GD9 using a low dose of LPS (25 μg/kg) to mimic bacterial infection in mice. In particular, we hypothesized that an inflammatory stimulus at this age will result in alterations in behaviors related to mood disorders. Moreover, we evaluated different candidate cellular and molecular mechanisms that could underlie these behavioral alterations. We focused on the hippocampus because this region is particularly sensitive to inflammatory insults (Depino et al., 2005) and plays an integral role in the modulation of emotion.

EXPERIMENTAL PROCEDURES

Animals

C57BL/6J (C57, The Jackson Laboratory, Bar Harbor, Maine, USA) mice were bred for several generations in the animal house at the Leloir Institute (Buenos Aires, Argentina), under non-SPF conditions. Each C57 nulliparous female was mated with a C57 male at 8–12 weeks of age during two nights, and then males were removed from the cage. The day between mating nights was considered the gestational day (GD) 0 for simplicity, and it was GD0.5 or GD-0.5 depending on the actual mating night. At GD9 pregnant mice were randomly assigned to either experimental group and injected subcutaneously with 25 μg/kg of LPS (*Escherichia coli* LPS, serotype 0111:B4, Sigma, St. Louis, MO, USA) or with sterile saline solution (Sal). The day of parturition was defined as postnatal day (PD) 0. To minimize the ''litter effect'', 9 Sal and 12 LPS litters were used and 1–2 pups per litter were randomly selected for behavioral or neurophysiological studies. We observed no effect of treatment on litter size or weight at weaning (data not shown). Animals were weaned at PD21 and housed four to five per cage. For behavior, 17 male offspring of each experimental group were studied (female mice were not included to reduce the number of independent variables evaluated, specially considering the effects of the estrous cycle on anxiety- and depression-related behaviors (Meziane et al., 2007)). To avoid the confounding effect of behavioral testing on biochemical and cellular measurements, untested littermates of tested animals were used for neurotransmitters, neurogenesis and Reelin-positive cells measurements.

All animals had water and food *ad libitum* and were housed on a 12:12 light/dark cycle with lights on at 0800 h. All animal procedures were performed according to EC Directive 86/609/EEC for animal experiments and the regulations for the use of laboratory animals of the National Institutes of Health, USA, and approved by the animal subjects review board (CICUAL) of the Leloir Institute.

Behavioral testing

Behavioral testing was performed during the light period (between 10:00 and 16:00 h) under dim light illumination. Mice were 8–10 weeks of age at the beginning of testing, and they were subjected to all tests using 1-week intervals to reduce inter-test interactions. Tests were performed in the order listed below, in the holding room. After changing illumination, mice were habituated to it for 45 min prior to the test. The investigator was blind to prenatal treatment during behavioral testing. After testing, each mouse was identified and placed in a holding cage until all animals in a cage were tested. Each apparatus was cleaned with 20% ethanol between sessions.

The elevated plus maze was performed as previously described (Depino et al., 2008, 2011; Lucchina et al., 2010). The maze consisted of two open and two closed arms (open arms: 30 × 5 cm, 105 lux, surrounded by a 0.5-cm high border; closed arms: 30 × 5 cm, 43 lux, surrounded by 19-cm high walls). The walls were made of black PVC and the floor of gray PVC. The apparatus was elevated 50 cm above the floor. Mice were placed into the central platform (5 × 5 cm, 102 lux) of the maze facing toward an open arm and allowed to explore the maze for 5 min. Locomotion data were collected by the ANY-maze system (Stoelting, IL, USA). Measured variables were: total distance, time and distance in the open and in the closed arms, and time and distance in the central platform.

The open field test was performed as previously described (Depino et al., 2008, 2011; Lucchina et al., 2010). Mice were placed in an arena (floor: 45 × 45 cm of gray PVC; walls: 30 cm high of black formic; 30 lux) for 5 min. Animals were initially placed along one side of the arena, and the center region was defined as the central 23 × 23 cm area. Locomotion data were collected by the ANY-maze system (Stoelting). Measured variables were: total distance, entries, distance and time in the center, latency to enter the center, time and distance in the periphery. We also calculated the percentage of distance in the center.

The light/dark test was performed as previously described (Depino et al., 2008; Lucchina et al., 2010; Campolongo et al., 2012). A 45-cm × 45-cm arena was divided into half with an inverted black box (lit side: 35 lux; dark side: 1 lux). Animals freely moved between

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