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Single early prenatal lipopolysaccharide exposure impairs striatal monoamines and maternal care in female rats

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ABSTRACT

Aims: Environmental information received by a mother can induce a phenotype change in her offspring, commonly known as a maternal effect (trans-generational effect). The present work verified the effects of lipopolysaccharide (LPS), which mimics bacterial infection, on maternal care and on the activity of related brain areas in F1 offspring, i.e., female rats that were prenatally exposed to LPS.
Main methods: Pregnant rats received 100 μg/kg of LPS intraperitoneally on gestational day (GD) 9.5. Female offspring of the F1 generation were mated to naïve males and were evaluated during their lactation period for open field, maternal and aggressive behaviors. Striatal and hypothalamic dopamine and serotonin levels and turnover were also evaluated. Furthermore, astrocyte protein expression in the nucleus accumbens (NA) was analyzed in F1 females to assess LPS-induced neuroinflammation.
Key findings: Prenatal LPS did not change open field behavior but impaired both maternal and maternal aggressive behaviors in the F1 generation. LPS exposure also reduced both striatal levels of dopamine and serotonin and its metabolites, but induced no changes in NA astrocyte expression.
Significance: We suggested that the observed impairments in the F1 females were a consequence of a motivational change induced by prenatal LPS, as (1) no changes in motor activity were observed, (2) prenatal LPS-exposure was reported by our group to induce motivational impairments in males, and (3) the existence of a strong connection between striatal dopaminergic activity and motivation-oriented activities. The present findings strongly indicate a maternal effect for prenatal LPS, at least for the F1 generation.

Introduction

Lipopolysaccharide (LPS), an endotoxin that originates from the cell wall of gram-negative bacteria, which mimics bacterial infection, activates the immune system to release proinflammatory cytokines (Avitsur et al., 1997; Saluk-Juszczak and Wachowicz, 2005). Viral and bacterial infections, including those caused by prenatal LPS exposure, induce short and long-term changes in behavior and central nervous system activity (Boksa, 2010; Golan et al., 2005; Meyer et al., 2009). Previous investigations by our group have shown that prenatal LPS (100 μg/kg, given intraperitoneally on gestational day [GD] 9.5) reduces the social behavior of F1 males both in infancy and in adulthood and decreases their striatal dopamine (DA) and DA metabolite levels in the absence of signs indicative of neuroinflammation (Kirsten et al., 2010a, 2010b, 2011). Interestingly, our model also showed that parental maternal behavior was slightly improved in pregnant rats treated with LPS on GD 9.5 (Kirsten et al., 2011), whereas treatment on GD 21 decreased this behavior (Bernardi et al., 2010).

It has been suggested that the effects of maternal LPS exposure on the developing fetal brain are not directly mediated by LPS, but are instead indirectly induced via increases in proinflammatory cytokines and glucocorticoid levels within the maternal circulation, placenta and fetal brain (Ashdown et al., 2006; Cai et al., 2000; Gayle et al., 2004; Urakubo et al., 2001). Infections associated with immunological events in the early/middle fetal stages (e.g., GD 8–10 in rats and mice) might have a stronger impact on neurodevelopment than late-stage pregnancy infections. Immune activation during the early/middle stages of pregnancy was shown to modify fetal cell proliferation and differentiation, cell migration, target selection, and synapse maturation (Ghiani et al., 2011; Meyer et al., 2006, 2007; Samuelsson et al., 2006; Shi et al., 2003). Multiple brain injuries and behavioral abnormalities persisting through adulthood, were also reported after early/middle stage pregnancy infections (Meyer et al., 2007).

Environmental information received by a mother can induce a phenotypic change in her offspring, commonly known as a maternal or trans-generational effect (Agrawal et al., 1999; Curno et al., 2009).

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Certain cues in the maternal environment, e.g., the prevalence of predators, or maternal infection can lead to behavioral, morphological and immunological changes in the following generation (Agrawal et al., 1999; Grindstaff et al., 2006).

The present experiment was designed to analyze possible LPS-induced effects (transgenerational effect) on the maternal care of the F1 generation, i.e., in adult female rats prenatally exposed to LPS (100 μg/kg LPS on GD 9.5). We determined the behavioral, neurochemical and neuroinflammatory outcomes related to maternal care in the F1 generation. Specifically, the following parameters were analyzed: maternal, aggressive and open field behaviors, striatal and hypothalamic DA and serotonin (5-HT) levels, levels of DA and 5-HT metabolites and turnover, and astrocyte expression in the nucleus accumbens (NA). Astrocytes that increase in number and become activated following an infection or an immunological challenge, such as an acute administration of LPS were considered indicative of neuroinflammation (Pang et al., 2010).

Material and methods

Animals

Forty-eight pregnant Wistar rats (parental generation) between 12 and 13 weeks of age and weighing 216–263 g were used to generate the F1 offspring (GD 0 was defined as the day when spermatozoa were detected in a vaginal smear). Pregnant dams were individually housed in polypropylene cages (38 × 32 × 16 cm) at a controlled temperature (22 ± 2 °C) and humidity (65–70%) with artificial lighting (12-hour light/12-hour dark cycle, lights on at 6:00 AM). The animals had free access to Nuvilab® rodent chow (Nuvital Co., Sao Paulo, SP, Brazil) and filtered water. Sterilized, residue-free wood shavings were used as bedding. The animals were divided into control (saline-treated) and experimental (LPS-treated) groups (n = 24 dams/group). The dams were allowed to give birth and nurture their offspring with no interference. The day of birth was recorded as postnatal day (PND) 1. No handling was performed on PND 1, but on PND 2, 8 pups (4 males and 4 females) from each litter were randomly selected. No cross-fostering procedures were used. Litters with fewer than 8 pups were culled. The 8 randomly selected pups remained with each dam until weaning (PND 21). On PND 21, littermates were separated and housed by sex under the same conditions as their parents. One adult female from each litter (F1 generation) was used for each experiments, using different animals in each experiment. Male pups were kept apart for use elsewhere (Kirsten et al., 2010a, 2010b).

As depicted in Fig. 1, on PND 90, adult female rats of the F1 generation were mated with experienced males from our colony. These female rats were not used in any other study. The gestational and neonatal procedures were the same as those described above for their parents. On lactation days (LD) 5 and 6 of the F1 generation, the following experiments were performed: open field behavior, maternal and aggressive behavior, neurochemistry and immunohistochemistry. The animals used in these experiments were kept in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources of Paulista University, Brazil (protocol No. 014/09, CEUA-UNIP). These guidelines are similar to those of the National Institutes of Health, Bethesda, MD. Experiments were carried out in accordance with good laboratory practice protocols and with quality assurance methods.

Treatment

LPS (from *Escherichia coli*, Sigma®, Saint Louis, MO, USA, serotype 0127: B8) was dissolved in sterile saline (50 μg/ml LPS in a 0.9% NaCl solution) and administered intraperitoneally to pregnant dams (parental generation) at a dose of 100 μg/kg on GD 9.5. This dose was chosen because it has been shown to (1) elicit sickness behavior, (2) induce endocrine alterations in dams, (3) increase cytokines at the placental level, (4) impair the offspring birth rate and (5) reduce the social behavior of male offspring during infancy and adulthood (Kirsten et al., 2010b; Spencer et al., 2007; Wang et al., 2006). The control group consisted of pregnant rats that received only sterile saline (0.9% NaCl) on the same treatment schedule as the LPS animals. Each control dam was treated with 0.1 ml/100 g saline solution.

General activity in the open field

The general activity test was performed on LD 5 of the F1 generation, i.e., in adult female rats prenatally exposed to LPS (n = 10 dams/group). The open field device consisted of a round wooden arena (90 cm in diameter with 28 cm high walls) that was painted gray and had a washable, acrylic covering (Broadhurst, 1960). For the observations, each rat was individually placed in the center of the apparatus and the following parameters were automatically measured using Ethovision software (Ethovision; Noldus Information Technology, Leesburg, VA) over a period of 5 min: total locomotor activity (traveled distance [cm]), time spent in locomotor activity (time movement [s]), mean velocity (cm/s), and rearing and grooming time (s) and frequencies. A video camera mounted 100 cm above the arena was used to collect the data that were analyzed by the Ethovision System® software which was installed on an IBM-compatible computer placed in an adjacent room. The arena was washed with a 5% alcohol/water solution before placement of the animals to obviate possible biasing effects from odor clues left by the previously tested rats. Control and experimental (F1) rats were intermixed for observations.

Maternal behavior

Maternal behavior testing was performed on LD 6 of the F1 generation, i.e., in adult female rats prenatally exposed to LPS (n = 10 dams/group). Pups were removed from their dams and placed in a different

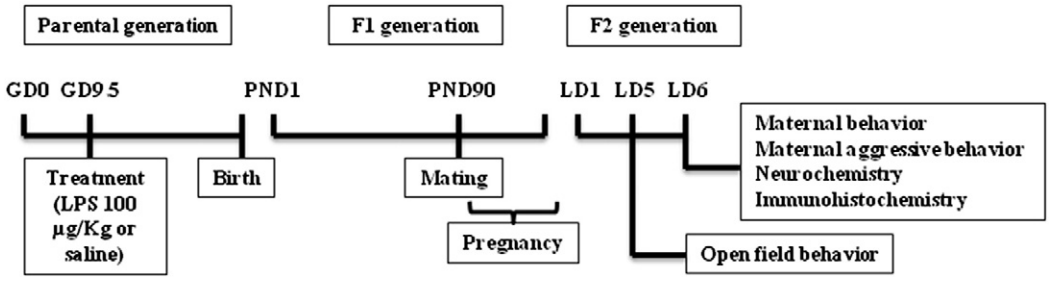

Fig. 1. Experimental design diagram. Gestational day: GD (parental generation). Postnatal day: PND (F1 generation). Lactation day: LD (F2 generation). All the behavioral and neurological tests were performed on the F1 generation.