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Hyperactive behavior in female rats *in utero*-exposed to group B *Streptococcus*-induced inflammation

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ABSTRACT

Group B *Streptococcus* (GBS) is one the most common bacterium responsible of maternal infections during pregnancy. Offspring *in utero*-exposed to GBS-induced placental inflammation displayed sex-specific forebrain injuries. Sex differences have been reported in several neuropsychiatric disorders. Hence, we hypothesized that female rats *in utero*-exposed to GBS may present sex-specific neurobehavioral impairments. Lewis rats were injected intraperitoneally every 12 h from gestational day (G) 19 to G22 with either saline (controls) or inactivated serotype Ia GBS (10^9 CFU). Before puberty, no difference in terms of spontaneous motor activity, exploratory or anxiety-related behaviors was noticed between experimental conditions. During puberty, GBS-exposed females – but not males – performed worse than same-sex controls in a forced motor task. During adulthood, GBS-exposed females – but not males – displayed increased spontaneous locomotor activity and decreased inhibition. In conclusion, our findings show for the first time that adult females – but not males – *in utero*-exposed to GBS-induced inflammation presented a hyperactive and disinhibited phenotype emerging after puberty.

1. Introduction

Clinical and preclinical data support a causal link between perinatal inflammation and brain injuries, contributing to various neuropsychiatric disorders (Mann and McDermott, 2011; Spencer and Meyer, 2017; Zavitsanou et al., 2014). Epidemiological studies have documented sex differences in the occurrence of many neurobehavioral disorders, for instance in autism, depression, or attention-deficit hyperactivity disorder (ADHD) (Ferri et al., 2018; Huang et al., 2016; Instanes et al., 2017; Rainville et al., 2017). The activation of multiple innate immune pathways during gestation has been shown to exert sex-specific effects on the fetal brain, associated with sex-dependent behavioral impairments (Rana et al., 2012).

Group B *Streptococcus* (GBS) is a bacterium colonizing the lower genital tract of 15–30% of pregnant women (Larsen and Sever, 2008). GBS infection throughout pregnancy can be transient, intermittent or persistent, leading to placental infection and/or inflammation (chorioamnionitis), urinary tract infection or, in the worst case, bacteremia or sepsis (Larsen and Sever, 2008). GBS is the most frequent bacterium causing chorioamnionitis at the end of gestation (Larsen and Sever, 2008).

Previous investigations documented sex-specific placental inflammatory responses leading to placental and brain injuries, and subsequent autistic-like behaviors in male rats *in utero*-exposed to live and inactivated serotype Ia GBS (Allard et al., 2017; Bergeron et al., 2016, 2013; Bucci et al., 2008; Rucklidge, 2010). Nevertheless, brain injuries were also present in female rats *in utero*-exposed to GBS-induced inflammation (Bergeron et al., 2013). Notably, a decreased thickness of the external capsule, a reduced periventricular myelin basic protein staining, oligodendrocyte maturation impairments, and a reduced density of microglial cells were detected in the periventricular white matter of GBS-exposed females (Bergeron et al., 2013). In line with these histological observations, some of the main brain networks involved in attention-deficit hyperactivity disorder (ADHD) are those connecting anterior and posterior attentional systems – such as the right superior longitudinal fasciculus and the inferior fronto-occipital fasciculus – passing through the external capsule and other periventricular white matter areas (Pastura et al., 2016). Young adult female rats *in utero*-exposed to GBS-induced inflammation displayed a hyper-social behavior during late puberty, as opposed to GBS-exposed males, which were hypo-social compared to same-sex controls (CTLs) (Bergeron et al., 2013). Interestingly, “social intrusiveness” is one of the diagnostic features of ADHD, which is a disorder characterized by a persistent pattern of inattention and/or hyperactivity-impulsivity (DSM-5

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Classification, 2013).

In line with our previous work and data from the literature, we hypothesized that females exposed prenatally to GBS-induced inflammation will display sex-specific neurobehavioral impairments. We based our experimental design on our already established rat model (Bergeron et al., 2016, 2013) of GBS-induced chorioamnionitis to investigate its sex-specific effect on neurobehavioral impairments.

2. Material and methods

The experiments were approved by the Institutional Animal Care and Use Committee of the *Université de Sherbrooke* (protocol #336-13B) in accordance with the Canadian Council on Animal Care guidelines. Pregnant primiparous Lewis rats (number (n) = 14) were obtained from Charles River Laboratories (Saint-Constant, Qc, Canada). Dams were individually housed in polypropylene opaque cages (47.0 × 25.0 × 14.5 cm) in a quiet and controlled room starting on gestational day (G) 14. They were reared on a 12 h light/dark cycle (lights on at 6 A M) at 20–23 °C with access to sterilized laboratory chow (Charles River Laboratories) and water *ad libitum*.

2.1. Experimental design

This study was designed based on previous findings from our team and aimed to further characterize the sex-specific behavioral alterations detected in a rat model of end-gestational exposure to inactivated GBS (Bergeron et al., 2016, 2013). Formaldehyde-killed serotype Ia GBS (reference strain A909) was used for all experiments. Dams were randomized into two groups and injected intraperitoneally every 12 h (8 A M/PM) from gestational day (G) 19 to G22 with 100 µL of either: sterile 0.9% saline (CTL) or serotype Ia GBS (10^9 CFU/100 µL of saline) (Fig. 1). Dams were weighed twice per day from G19 to G22 (eight CTL dams, six GBS Ia dams). Two dams from each experimental group underwent cesarean-sections to validate the presence of histological chorioamnionitis we previously documented in this experimental design (Bergeron et al., 2016, 2013). Dams were regularly monitored until delivery (G23, or postnatal day (P) 0) to detect any sickness-related behavior or premature deliveries. Pups were counted and weighed daily from P1 to prevent any stressful situation by moving them on P0. Our behavioral experiments were carried out in three age-periods: prepuberty (P15-25; open field), late puberty (P30-50; rotarod and elevated-plus-maze) and adulthood (P105-110; elevated-plus-maze) (Kállai et al., 2017). Each rat offspring was not utilized for more than three distinct behavioral tests through lifespan. After the open field test, subjects were split into two groups: one group was performing the rotarod test (P30, P35, and P40) and one group was performing the elevated-plus-maze test (P40-P50). The animals were tested during the morning (8:00 – 11:00 AM) for all behavioral tests.

2.2. Open field exploratory activity (Prepuberty (P15, P20, and P25))

The open field test was performed to evaluate spontaneous locomotion, exploratory behavior and anxiety longitudinally at P15, P20 and P25, as previously described (Allard et al., 2017; Bergeron et al., 2013). Rats were allowed to habituate to testing room in for 30 min prior to testing in the open field apparatus (40 × 40 × 40 cm dimensions). Automatic tracking and recording (Any-Maze Video Tracking System, Stoelting Co, Wood Dale, IL, USA) of freely moving animals in the enclosure – separated in 16 equal virtual squares – for 5 min was started as soon as the rat was placed in the corner, facing the central zone. The apparatus was cleaned between each rat. Parameters analyzed were: total distance travelled in the apparatus, duration of mobility, number of visited squares, number of virtual lines crossed, average speed (total distance travelled divided by time of mobility), and thigmotaxis (*i.e.* an index of anxiety in rodents reflected by a tendency to remain close to the walls) in a novel environment reflected by time spent in the central zone (the four central virtual squares) (Simon et al., 1994). The number of rats tested in the open field was: CTL female (n = 13), GBS female (n = 10), CTL male (n = 7), GBS male (n = 10), from six CTL and four GBS Ia litters.

2.3. Rotarod (Late puberty (P30, P35, and P40))

The accelerating rotarod was used to assess motor learning, motor coordination and balance (Majdak et al., 2016; Söderlund et al., 2015). The subjects were longitudinally assessed at P30, P35, and P40, as described (Bergeron et al., 2013). Male rats were acclimatized to experimentation room 30 min prior to testing and were assessed first while the females remained in the housing room. Then, female rats were transported from housing room to habituate to the experimentation room, and subsequently underwent the rotarod test. Briefly, six trials per rat were conducted on each day. Rats were placed on a rod (60 mm of diameter, 75 mm in length) rotating at a speed of 4 rotations per minute (rpm) that gradually increased from 4 to 40 rpm over 1 min. Tested rats were placed in their home cage for 2 min between each trial. The apparatus was cleaned between each trial. The latency to fall (seconds) and the rod speed value (rpm) were automatically recorded. Parameters measured were the longest latency to fall (*i.e.* the best trial), the latency to fall and the rod speed when falling at each trial (six trials per day). The number of rats tested for the rotarod test was: CTL female (n = 12), GBS female (n = 4), CTL male (n = 5), GBS male (n = 4), from six CTL and four GBS Ia litters.

2.4. Elevated-plus-maze (Late puberty (P40-P50), and adulthood (P105-P110))

Anxiety-related behavior and locomotion were evaluated using the elevated-plus-maze (EPM) at a young adult (P40-P50) and adult (P105-P110) age. The EPM is a cross-shaped apparatus elevated at 50 cm from the floor, including two open arms and two opaque enclosed arms 40 cm of height. Rats normally display a preference for the walled (closed) arms, the open arms being more aversive for them. Rats were allowed to habituate to the dimly illuminated experimentation room for 30 min prior to testing. Male rats were tested before female rats. The apparatus was cleaned and dried between each rat. Automatic tracking and recording of freely moving animals (Any-Maze Video Tracking

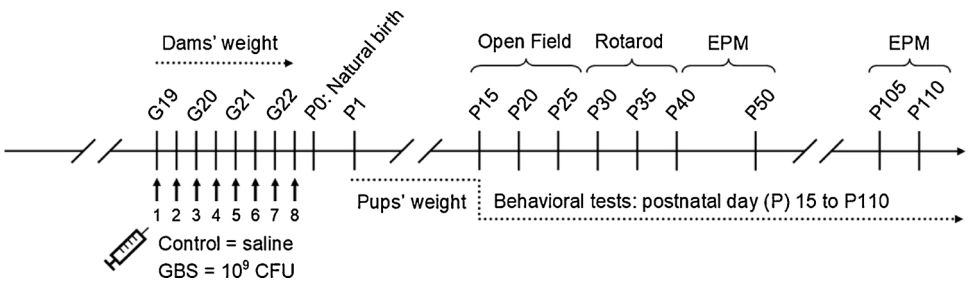

Fig. 1. Treatment administration and behavioral experiment scheme. Abbreviations: EPM: Elevated-plus-maze; G: Gestational day; GBS: Group B *Streptococcus*.