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Additive effects of maternal iron deficiency and prenatal immune activation on adult behaviors in rat offspring

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ABSTRACT

Both iron deficiency (ID) and infection are common during pregnancy and studies have described altered brain development in offspring as a result of these individual maternal exposures. Given their high global incidence, these two insults may occur simultaneously during pregnancy. We recently described a rat model which pairs dietary ID during pregnancy and prenatal immune activation. Pregnant rats were placed on iron sufficient (IS) or ID diets from embryonic day 2 (E2) until postnatal day 7, and administered the bacterial endotoxin, lipopolysaccharide (LPS) or saline on E15/16. In this model, LPS administration on E15 caused greater induction of the pro-inflammatory cytokines, interleukin-6 and tumor necrosis factor-α, in ID dams compared to IS dams. This suggested that the combination of prenatal immune activation on a background of maternal ID might have more adverse neurodevelopmental consequences for the offspring than exposure to either insult alone. In this study we used this model to determine whether combined exposure to maternal ID and prenatal immune activation interact to affect juvenile and adult behaviors in the offspring. We assessed behaviors relevant to deficits in humans or animals that have been associated with exposure to either maternal ID or prenatal immune activation alone. Adult offspring from ID dams displayed significant deficits in pre-pulse inhibition of acoustic startle and in passive avoidance learning, together with increases in cytochrome oxidase immunohistochemistry, a marker of metabolic activity, in the ventral hippocampus immediately after passive avoidance testing. Offspring from LPS treated dams showed a significant increase in social behavior with unfamiliar rats, and subtle locomotor changes during exploration in an open field and in response to amphetamine. Surprisingly, there was no interaction between effects of the two insults on the behaviors assessed, and few observed alterations in juvenile behavior. Our findings show that long-term effects of maternal ID and prenatal LPS were additive, such that offspring exposed to both insults displayed more adult behavioral abnormalities than offspring exposed to one alone.

1. Introduction

Iron deficiency (ID) is a common and continuing public health issue during pregnancy and early childhood. An estimated 30% of women worldwide are iron deficient, and this percentage increases to a global incidence of 42% when considering ID during pregnancy (McLean et al., 2009). ID during pregnancy is associated with an increased risk of preterm birth and low birth weight, which increases the risk of disease during development and adulthood (Gambling et al., 2003; Haider et al., 2013; Radlowski and Johnson, 2013). Maternal ID also places the developing fetus at risk for neuronal changes, as iron is integral for myelination, mitochondrial efficiency and neurotransmitter metabolism (Connor and Menzies, 1996; Hare et al., 2013). Indeed maternal and early life ID are associated with reductions in motor skills, learning and cognition, increases in anxiety, depression, social problems and attentional alterations in childhood and adolescence, and in the risk for development of schizophrenia (Lozoff et al., 2006; Insel et al., 2008; Sorensen et al., 2010). Animal models of pre- and peri-natal ID have shown reductions and delays in myelination (Yu et al., 1986; Wu et al., 2008), altered brain monoamine content (Coe et al., 2009), and alterations in hippocampal size, neurochemistry and activity (de Deungria et al., 2000; Rao et al., 2011) as well as persistent deficits in sensorimotor and hippocampal-dependent behaviors (Kwik-Uribe et al., 2000; Felt et al., 2006) and increased anxiety and exploratory behavior (Eseh and Zimmerberg, 2005) in offspring.

Similar to maternal ID, infections during pregnancy are varied and prevalent in developing and developed countries (Velu et al.,

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2011) and are associated with an increased risk of adverse birth outcomes (Rasmussen et al., 2012), cerebral palsy (Miller et al., 2013), and juvenile and adult onset psychiatric disorders such as schizophrenia and autism (Brown, 2006, 2011; Khandaker et al., 2013). Animal models of prenatal immune activation have been used widely to model the effects of bacterial endotoxins, viral mimics, and peripheral infections on brain development and behavior. These studies have described structural and neurochemical changes in the brains of offspring, as well as significant behavioral alterations during development, adolescence and adulthood, including deficits in pre-pulse (PPI) inhibition of acoustic startle, increased sensitivity to amphetamine, and reductions in associative and spatial learning (for review see Boksa, 2010).

Prenatal immune activation and maternal ID can individually affect neurodevelopment and health in later life, however, the interaction between these insults may be more significant. Exposure to a bacterial immune challenge during a state of active ID results in a potentiation of the pro-inflammatory cytokine response (Pagani et al., 2011). In turn, this enhanced cytokine induction results in a further decrease in serum iron (Kemna et al., 2005). The mechanisms of this circular relationship are still being investigated in non-pregnant animals, however, our objective was to understand the consequences of any interactions between these factors on a pregnant animal and her offspring.

Therefore, given the prevalence of these two environmental factors during pregnancy, their associated effects on brain development, as well as the circular relationship between ID and inflammation, we recently described a rat model which pairs maternal ID during pregnancy and prenatal immune activation (Harvey and Boksa, 2014). Pregnant rat dams were placed on an ID diet from embryonic day 2 (E2) until postnatal day 7 (P7), and were also administered the bacterial endotoxin, lipopolysaccharide (LPS), on E15 and 16 to induce a state of prenatal immune activation. After just 13 days on an ID diet (E15), we confirmed that dams showed a significant 40% reduction in serum iron and 20% reduction in placental iron compared to dams on an iron sufficient (IS) diet (Harvey and Boksa, 2014). LPS administration on E15 induced an increase in serum levels of pro-inflammatory cytokines, interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), in these dams. Most interestingly, we demonstrated that there was an interaction between maternal ID and prenatal immune activation, as there was significantly greater induction of IL-6 and TNF-α in response to LPS in ID dams compared to IS dams (Harvey and Boksa, 2014). Since IL-6 is thought to be one of the key mediators driving the alterations in brain development and behavior in animals as a result of prenatal immune activation (Smith et al., 2007), this suggested that the combination of prenatal immune activation on a background of maternal ID might have more adverse consequences than exposure to either insult alone.

In this combined maternal ID/prenatal LPS model, the consequences of maternal ID on the offspring were significant. Offspring from ID dams showed 75–85% reduction in spleen and liver iron at P7 and 50% reduction in serum iron at P21, however, the increase in IL-6 in ID LPS dams did not result in more severe ID in the ID LPS offspring (Harvey and Boksa, 2014). Importantly, brain iron content of offspring from ID dams was 65% of control at P7 (Harvey and Boksa, 2014). Neurodevelopmental screening of perinatal offspring (P6–P18) in this combined model showed that pups from ID dams displayed abnormalities in forelimb grasp and acoustic startle, while pups from LPS treated dams displayed differences in grip ability, geotaxis reflex, cliff avoidance and acoustic startle (Harvey and Boksa, 2014). However, we did not observe any interaction between the two insults with respect to the markers of early neurodevelopment examined. Instead, our findings showed that the two insults produced an additive phenotype; offspring exposed to both maternal ID and prenatal LPS displayed the sum of neurodevelopmental abnormalities produced by either factor alone. However, while we did not observe any interaction on markers of early neurodevelopment, we did not test more complex juvenile or adult behaviors of learning, cognition and attention which have previously been shown, in separate models of maternal ID or prenatal immune activation, to be altered by these early environmental insults.

Thus, the aim of this study was to use our previously described rat model to determine whether there is an interaction between maternal ID and prenatal immune activation that alters and potentiates abnormalities in juvenile and adult behaviors in the offspring. We assessed a variety of behaviors chosen because of their relevance to described deficits in humans as a result of maternal and early life ID or prenatal infection. We assessed passive avoidance behavior as a measure of learning and memory and also measured a metabolic marker of neuronal activity in the brain following passive avoidance testing. We assessed pre-pulse inhibition of acoustic startle, amphetamine induced locomotion, social interaction and sensitivity to novelty, which are behaviors previously shown to be altered in some rodent models of prenatal immune activation and are relevant to neurodevelopmental psychiatric disorders such as schizophrenia and autism. Finally, in light of locomotor deficits we described previously in this model (Harvey and Boksa, 2014), we assessed motor performance, as well as exploratory activity in an open field. We hypothesized that maternal ID and prenatal LPS administration would interact to significantly alter behavior in juvenile and adult offspring, resulting in a more severe behavioral phenotype than the phenotype resulting from either prenatal insult alone.

2. Methods

2.1. Animals

Timed pregnant Sprague Dawley rats were obtained from Charles River at E2 and housed individually at 21 ± 2 °C with a 12 h light/dark cycle (lights on at 8:00 a.m) and with food and water ad libitum. Animals were treated in accordance with guidelines from the Canadian Council on Animal Care (www.ccac.ca) and protocols approved by the McGill University Animal Care Committee.

2.1.1. Dietary manipulation of iron intake

On E2, pregnant dams were placed on either an ID (background iron 3–8 ppm) or IS (200 mg/kg) modified AIN-93G diet (Harlan Teklad Diets, Madison WI, USA). Dams continued on their respective diets throughout pregnancy, until P7, at which time all dams were placed on Global 18% Protein Diet (iron content, 200 mg/kg, Harlan Teklad Diets).

2.1.2. LPS administration to pregnant rats

On E15 and E16, dams on IS and ID diets received an injection of LPS (50 μg/kg, i.p., *Escherichia coli* serotype 0111:B4, Lot 42k4120, Sigma–Aldrich, Canada) or 0.9% saline (4 ml/kg, i.p.), between 10:00 a.m. and noon. Pregnancies were allowed to continue as normal. Previous experiments in our laboratory have shown that dosages of 50–100 μg/kg LPS administered between E15–E18 reliably induce fever, production of pro- and anti-inflammatory cytokines and increases in serum corticosterone in the dam (Ashdown et al., 2007; Cui et al., 2009, 2011; Harvey and Boksa, 2014). We have shown that LPS administered i.p. at E18 reaches the placenta, but not the fetus, and induces a pro-inflammatory cytokine response in the placenta but not in fetal brain or liver (Ashdown et al., 2006).