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Environmental enrichment mitigates the sex-specific effects of gestational inflammation on social engagement and the hypothalamic pituitary adrenal axis-feedback system

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ABSTRACT

Modest environmental enrichment (EE) is well recognized to protect and rescue the brain from the consequences of a variety of insults. Although animal models of maternal immune activation (MIA) are associated with several neurodevelopmental impairments in both the behavioral and cognitive functioning of offspring, the impact of EE in protecting or reversing these effects has not been fully evaluated. In the present study, female Sprague–Dawley rats were randomized into EE (pair-housed in a large multi-level cage with toys, tubes and ramps) or animal care control (ACC; pair-housed in standard cages) conditions. Each pair was bred, following assignment to their housing condition, and administered 100 μg/kg of lipopolysaccharide (LPS) on gestational day 11. After birth, and until the end of the study, offspring were maintained in their respective housing conditions. EE protected against both the social and hypothalamic pituitary adrenal axis consequences of MIA in juvenile male rats, but surprisingly not against the spatial discrimination deficits or accompanying decrease in glutamate levels within the hippocampus (as measured via LCMS–MS). Based on these preliminary results, the mechanisms that underlie the sex-specific consequences that follow MIA appear to be dependent on environmental context. Together, this work highlights the importance of environmental complexity in the prevention of neurodevelopmental deficits following MIA.

1. Introduction

Programming cues such as physical or psychogenic stressors, experienced during gestation, have a far reaching impact on general health (Lipkind et al., 2010; Nugent et al., 2011; see Pembrey, 2002; Soberanes-Chávez et al., 2013) and behavioral/cognitive functioning (Brown, 2012; Brown and Derkits, 2010; Mueller and Bale, 2008). Preclinical studies are particularly suggestive of the involvement of maternal immune activation (MIA) in later social and cognitive impairments of offspring (see Patterson, 2009; Meyer et al., 2007). For example, MIA has been associated with impaired object recognition (Coyle et al., 2009; Graciarena et al., 2010) and spatial memory (Golan et al., 2005; Howland et al., 2012), decreased prepulse (Fortier et al., 2007; Wolff and Bilkey, 2010) and latent (Bitanihirwe et al., 2010; Meyer et al., 2006) inhibition, increased amphetamine induced locomotion (Fortier et al., 2004; Poinkewitz et al., 2011), and altered social behaviors (Bitanihirwe et al., 2010; Hava et al., 2006; Malkova et al., 2012). Additionally, in animal models of MIA, the associated brain changes are reported to parallel the pathogenesis of schizophrenia and autism including diminished NMDA receptor functioning, imbalances in dopamine regulation, reductions in the glycoprotein reelin, and general immune dysregulation (Coyle et al., 2003; Meyer et al., 2008; see Patterson, 2009; Shi et al., 2003). Moreover, the behavioral impairments observed in offspring following MIA may be reversed by schizophrenia drug treatments (Shi et al., 2003). However, it is rare that studies investigate interventions that may protect against these neurodevelopmental effects of MIA.

Animals models of MIA utilize pathogens such as gram negative bacteria (lipopolysaccharide; LPS) and viruses (polyinositic:polycitidylic acid; polyI:C) to activate Toll-like receptors located on immune cells distributed throughout the brain and periphery (reviewed in Meyer et al., 2007). Lipopolysaccharide (LPS) mimics Gram negative infection by activating Toll-like receptor 4, which in turn initiates intracellular signaling events that lead to the synthesis of a variety of pro-inflammatory molecules such as

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interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α (Blatteis et al., 2004; Dinarello, 1999; Steiner et al., 2006). These molecules activate the central nervous system and *de novo* synthesis of cytokines within the brain that are responsible for the initiation of fever and its accompanying constellation of behavioral and cognitive disruptions (i.e., sleep changes, lethargy, performance disruptions on memory tasks, reduced food and water intake, etc.), known as sickness behaviors (Maier et al., 1998). In animal models of MIA, these behavioral and physiological markers can be used to verify the initiation of the sickness response (French et al., 2013), without stressing the mother by collecting serological samples to confirm inflammation. When evaluating gestational interventions that may mitigate the effects of MIA, such confirmation is necessary to determine if their success may be due to a direct prevention of sickness in the mother.

In contrast to the detrimental effects of MIA, environmental enrichment (EE) has been shown to improve performance on a variety of learning and memory measures such as object recognition (Leger et al., 2012) and spatial recall (Huang et al., 2006) tasks, and has even been reported to enhance social play behavior (Morley-Fletcher et al., 2003; Schneider et al., 2006). In the laboratory, EE typically takes the form of a larger home cage filled with novel toys of varying sizes, shapes and textures, which increase an animal's opportunity for exploration and activity. This housing environment has also been attributed to the prevention and reversal of the detrimental effects of a variety of challenges to the adult nervous system. For example, EE has been reported to delay motor deficits in an animal model of Huntington's Disease (Spires et al., 2004), rescue sensorimotor and spatial memory impairments after stroke (Dahlqvist et al., 2004; Ohlsson and Johansson, 1995), attenuate inflammation and the associated physiological and cognitive disruptions that accompany infection (Jurgens and Johnson, 2012; Kentner et al., 2008), and prevent functional deficits following traumatic brain injury (Johnson et al., 2013). There is also a report that EE counteracts the decreased social exploration and latency that accompanies prenatal valproic acid exposure (Schneider et al., 2006), which attenuates proinflammatory cytokines and reactive oxygen species (Cardinale et al., 2010). However, to-date, studies evaluating the effects of EE following early-life insults have focused on maternal separation (Francis et al., 2002; Vivinetto et al., 2013) and prenatal stressors such as restraint (Li et al., 2012; Morley-Fletcher et al., 2003) or early exposure to drugs of abuse/misuse (i.e., nicotine; Mychasiuk et al., 2014), but not MIA.

Therefore, in the present study we employed an early gestational LPS challenge and (1) confirmed MIA by passively quantifying sickness behaviors in the dam, and (2) assessed the protective effects of combined pre- and post-weaning EE against MIA-induced social and cognitive disruptions and (3) their associated markers of hypothalamic pituitary adrenal (HPA) axis and glutamatergic functioning in her juvenile male and female offspring.

2. Materials and methods

All experimental procedures were carried out in accordance with recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the study protocol approved by the Institutional Animal Care and Use Committee at MCPHS.

2.1. Animals and housing

Male and female Sprague–Dawley rats were obtained from Charles River (Wilmington, MA) and housed at 20 °C on a 12 h light/dark cycle (0700–1900 light) with food and water available *ad libitum*. Upon arrival, female rats were pair-housed in either EE (large multi-level cage with toys changed 2 times weekly, tubes, chew bone, Nestlets© and ramps; Critter Nation, Muncie IN) or Animal Care Control (ACC; standard cage with tube, chew bone, and Nestlets©) housing. Male rats were pair-housed in standard conditions. Rats were differentiated by a non-scented/non-toxic colored marking on their tail. A flowchart of the study procedures can be found in Fig. 1.

2.2. Breeding

Following one-week of acclimatization, estrous cycles were evaluated daily (Marcondes et al., 2002) for an additional week in order to determine individual cycle length for later pregnancy verification. During proestrous, a male rat was introduced to females in a 2:1 female:male ratio. The next morning vaginal samples were collected and breeding (gestational day 1) established by the presence of both spermatozoa and vaginal cells typical of estrus. Male breeders were then returned to their home cage. Pregnancy was confirmed by observations of the diestrus phase from vaginal samples, continued weight gain of more than 3–5 g/day, and visible teats during the later phase of gestation.

2.3. Gestational treatment and sickness evaluation

On the morning of gestational day (G)11 (between 9:00 and 10:00 h), ACC dams were separated into individual clean cages so that we could accurately measure food and water intake, and other sickness behaviors, following lipopolysaccharide (LPS) administration. EE dams were separated from their partner by a physical divider for the same reason. The physical divider (build directly into the EE cage) allowed for olfactory, auditory, and some tactile and visual contact, important components of EE conditions. Between 11:30 and 12:30 h, each dam was given an i.p. injection of either LPS (*Escherichia coli*, serotype 026:B6; L-3755, Sigma, St. Louis, MO; 100 μg/kg) in pyrogen-free saline, or an equivalent volume of pyrogen-free saline. The LPS injection produces a short-lived inflammatory response (Steiner et al., 2006). For each housing pair, one dam received LPS and the other received saline.

In order to confirm the sickness-inducing effects of gestational LPS, sickness behaviors were evaluated. Dams were passively assessed on measures of ptosis (droopy eyelids), piloerection (ruffled, unkempt coat appearance), and lethargy by an observer blind to the drug treatment. Each behavior was scored on a three point scale (none = 0, mild = 1, or severe = 2), as previously described (Kentner et al., 2006, 2007) at 60, 90, 105, 120 and 210 min post injection. Based on the presence and severity of these behaviors, rats were assigned a total composite sickness score at each time point (adapted from Hayley et al., 2002; Kentner et al., 2007) as follows: 0 = 'no sickness' indicated by all behaviors being scored as none, or one behavior scored as mild; 1 = two or more mild scores, no severe scores (i.e., mild lethargy and ptosis and/or piloerection); 2 = severe score on one sickness behavior (mild or none scored on all other behaviors); 3 = severe score on two sickness behaviors; 4 = 'very sick appearance' as indicated by 3 severe scores (severe ptosis, lethargy and piloerection). Anorectic effects were evaluated by weighing food and water bottles immediately before LPS treatment, and again 20 h later, in order to determine food and water intake. Body weights were also evaluated 20 h after injection at which point dams were reunited with their cage-mate in their respective housing assignments.

2.4. Parturition, litter phenotype and maternal behavior

On G19, ACC dams were again separated into individual cages where they stayed alone with their litters until weaning. EE female