Int. J. Devl Neuroscience 39 (2014) 68–78

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Sexually dimorphic effects of prenatal exposure to propionic acid and lipopolysaccharide on social behavior in neonatal, adolescent, and adult rats: Implications for autism spectrum disorders

Kelly A. Foley[a,b,*], Derrick F. MacFabe[b,d], Alisha Vaz[b], Klaus-Peter Ossenkopp[a,b,c], Martin Kavaliers[a,b,c]

[a] Graduate Program in Neuroscience, The University of Western Ontario, London, ON N6A 5B7, Canada
[b] Department of Psychology, The University of Western Ontario, London, ON N6A 5C2, Canada
[c] The Kilee Patchell-Evans Autism Research Group, Department of Psychology, The University of Western Ontario, London, ON N6A 5C2, Canada
[d] The Kilee Patchell-Evans Autism Research Group, Departments of Psychology and Psychiatry, Division of Developmental Disabilities, The University of Western Ontario, London, ON N6A 5C2, Canada

ARTICLE INFO

Article history:
Received 11 January 2014
Received in revised form 7 April 2014
Accepted 7 April 2014

Keywords:
Microbiota
Short chain fatty acid
Maternal immune activation
Sex differences
Neurodevelopmental disorders
Social interaction

ABSTRACT

Emerging evidence suggests that the gut microbiome plays an important role in immune functioning, behavioral regulation and neurodevelopment. Altered microbiome composition, including altered short chain fatty acids, and/or immune system dysfunction, may contribute to neurodevelopmental disorders such as autism spectrum disorders (ASD), with some children with ASD exhibiting both abnormal gut bacterial metabolite composition and immune system dysfunction. This study describes the effects of prenatal propionic acid (PPA), a short chain fatty acid and metabolic product of many antibiotic resistant enteric bacteria, and of prenatal lipopolysaccharide (LPS), a bacterial mimetic and microbiome component, on social behavior in male and female neonatal, adolescent and adult rats. Pregnant Long–Evans rats were injected once a day with either a low level of PPA (500 mg/kg SC) on gestation days G12–16, LPS (50 μg/kg SC) on G12, or vehicle control on G12 or G12–16. Sex- and age-specific, subtle effects on behavior were observed. Both male and female PPA treated pups were impaired in a test of their nest seeking response, suggesting impairment in olfactory-mediated neonatal social recognition. As well, adolescent males, born to PPA treated dams, approached a novel object more than control animals and showed increased levels of locomotor activity compared to prenatal PPA females. Prenatal LPS produced subtle impairments in social behavior in adult male and female rats. These findings raise the possibility that brief prenatal exposure to elevated levels of microbiome products, such as PPA or LPS, can subtly influence neonatal, adolescent and adult social behavior.

1. Introduction

Attention has increasingly focused on how host gut microbial populations, collectively known as the microbiome, influence health and disease. Through communication with the central and peripheral nervous system, modification in the various components of the microbiome have the potential to contribute to gastrointestinal (GI), immune, and neuropsychiatric disease (Cryan and Dinan, 2012; Nicholson et al., 2012; Stilling et al., 2014). Results of recent studies with germ-free mice have demonstrated that alterations in the GI microbiome are associated with changes in early gene expression, neurotransmitter turnover, stress response, immune function, as well as reduced social behavior (e.g., Desbonnet et al., 2013; Foster and Neufeld, 2013; Heijtz et al., 2011). Furthermore, alterations in the GI microbiome have been recently observed in a maternal immune activation (MIA) mouse model of autism spectrum disorders (ASD) (Hsiao et al., 2013).

There is mounting evidence that alterations in the composition of the microbiome and its metabolic products may contribute to

* Corresponding author at: Department of Psychology, Graduate Program in Neuroscience, Social Science Centre, Room 7418, The University of Western Ontario, 1151 Richmond St., London, ON N6A5C2, Canada. Tel.: +1 519 661 2111x81214; fax: +1 519 661 3961.
E-mail addresses: kellyafoley@gmail.com (K.A. Foley), dmacfabe@uwo.ca (D.F. MacFabe), avaz3@uwo.ca (A. Vaz), ossenkop@uwo.ca (K.-P. Ossenkopp), kavalier@uwo.ca (M. Kavaliers).

http://dx.doi.org/10.1016/j.ijdevneu.2014.04.001
0736-5748/

the development and/or maintenance of ASD in children (de Theije et al., 2014; El-Ansary et al., 2013; Hsiao et al., 2013; Williams et al., 2011). ASD are a broad range of neurodevelopmental disorders of unclear etiology and are behaviorally diagnosed, with impairments in verbal and social communication, social behavior, sensory functioning, and stereotyped and repetitive behavior (DiCicco-Bloom et al., 2006). There are a number of comorbid traits in ASD, including a subset of patients that have gastrointestinal (GI) symptoms which can include increased permeability/inflammation of the intestinal tract, altered carbohydrate metabolism, and unique bacterial populations (Horvath and Perman, 2002; Williams et al., 2011). Indeed, the severity of autistic symptoms has been associated with severity of GI dysfunction in some patients (Adams et al., 2011).

Abnormal levels of bacteria flora, including altered *Clostridia*, *Bacteroidetes*, and *Desulfovibrio* subtypes, have been found in the GI tract of autistic children (Finegold et al., 2012; Parracho et al., 2005; Williams et al., 2011). Metabolic products of these include the short chain fatty acids (SCFA) (Finegold et al., 2010) which at physiological levels are essential for normal and immune associated functions (Al-Lahham et al., 2010; Arpaia et al., 2013; Belkaid and Hand, 2014; De Vadder et al., 2014; Heijtz et al., 2011), but at higher levels may alter immune function and/or exacerbate ASD related behaviors.

The SCFA, propionic acid (PPA), produced by enteric bacteria as a fermentation product of carbohydrate and some protein metabolism, has been proposed as a potential environmental factor in the development of ASD (see MacFabe, 2012, 2013 for review). Elevated levels of PPA characterize the neurodevelopmental metabolic disorder propionic acidemia (Feliz et al., 2003), with Al-Owain et al. (2013) recently reporting a case study of propionic acidemia and ASD comorbidly. Additionally, elevated fecal levels of SCFA have been found in children with ASD (Wang et al., 2012). Central and peripheral administrations of PPA in male rats have produced a number of brain and behavioral changes consistent with ASD (Frye et al., 2013; MacFabe et al., 2007, 2008, 2011; MacFabe, 2012; Shultz et al., 2008; Thomas et al., 2010, 2012), with results of a recent study demonstrating developmental delay and alterations in locomotor activity and anxiety-like behavior in male and female adolescent rats following prenatal PPA administration (Foley et al., 2014). PPA may exert its effects through a variety of modes including; neurotransmitter synthesis/release, G-coupled receptor activation, immune dysregulation, inflammation, oxidative stress, altered lipid profiles, mitochondrial dysfunction, and epigenetic actions through inhibition of histone deacetylase, all of which have been associated with neurodevelopmental disorders including specifically that of ASD (Frye et al., 2013; Inoue et al., 2012; MacFabe, 2012, 2013).

Prenatal administration of PPA in rats to date has been based on the valproate model of ASD. PPA, similar to valproate, is capable of acting as a histone deacetylase inhibitor to elicit epigenetic changes in gene expression, and both are fatty acids that can interfere with mitochondria cell metabolism (MacFabe, 2012; Frye et al., 2013). VPA is a common antiepileptic drug and with use in pregnancy, there is a risk for congenital malformations (e.g., spina bifida) and for ASD to develop in children, specifically when used in the first trimester (Bromley et al., 2008). Animal studies administering valproate during a comparable window of vulnerability (gestation day 12 in rats) produce offspring that display physical malformations, developmental delay, and behavioral deficits (reviewed in Roullet et al., 2013).

Several studies have linked maternal infections or inflammation during pregnancy to the development of ASD (Patterson, 2011; Zerbo et al., 2013). An immune insult during critical periods, and the accompanying release of proinflammatory cytokines acting both peripherally and centrally, may have adverse consequences for neurodevelopmental processes, such as cell differentiation, migration, and synaptogenesis (Bilbo and Schwarz, 2012; Patterson, 2011).

MIA in rodents, using a number of agents to induce an inflammatory response (e.g., the viral mimetic, polyinosinic:polycytidylic acid (poly I:C) and the bacterial mimetic, lipopolysaccharide (LPS)), has been used to investigate the role of the immune system in various behavioral disorders, including ASD. LPS is not only the major component of the cell wall of Gram-negative bacteria but is also a by-product of metabolism of many enteric bacteria. Offspring of dams treated with these immune agents display behavioral deficits in exploratory behavior and social interaction (Hsiao et al., 2013; Malkova et al., 2012; Smith et al., 2007). Hsiao et al. (2013) further reported that MIA produced alterations in the GI microbiome, suggesting a link between the immune and GI systems in this rodent model of ASD. Prenatal treatment with other environmental agents, such as valproate, which shares pharmacological properties with PPA (Silva et al., 2008; Thomas et al., 2010), also produces alterations in social behavior in adolescent and adult rats (Kim et al., 2011; Schneider and Przewlocki, 2005).

Impairments in social behavior have been reported following acute central administrations of PPA in male adolescent and adult rats, both in social interaction and social approach (MacFabe et al., 2007, 2011; Shultz et al., 2008), but to date, there have been no investigations of the potential effects of prenatal PPA on social behavior. Likewise, there are also relatively few reports on the effects of prenatal LPS on social behavior in either adult or, in particular, adolescent male and female rats. LPS administered in early or mid-late gestation resulted in decreased social play behavior and interaction in male adolescent and adult rats (Taylor et al., 2012; Kirsten et al., 2010).

The present study investigated the effects of prenatal treatment with either LPS or PPA on social and related behavior in male and female neonatal, adolescent, and adult rats. LPS and PPA were used to determine whether subtle changes in the components of the microbiome and its metabolic products can affect neurodevelopment. Following systemic administration of prenatal LPS and prenatal PPA, a variety of social behavior measures were assessed throughout the lifespan of male and female offspring. It was hypothesized that prenatal LPS and PPA would modify social behavior in adolescent and adult rats.

2. Materials and method

2.1. Animals

Female Long–Evans rats (230–305 g) were mated with adult males (370–575 g, Charles River, Canada) resulting in 16 litters. Females were paired with a male the night before behavioral estrus. Sperm present on a vaginal smear (hematoxylin and eosin stain) the next morning indicated successful mating and this was designated as gestational day 0 (G0). Dams were housed individually in standard polypropylene cages (45 cm × 22 cm × 20 cm) with ad libitum access to both food (ProLab RMH 3000) and water. A 12/12 h light–dark cycle (lights on at 07:00 h) was maintained in a temperature controlled colony room (21 ± 2 °C). Litters were born on G22 (designated as postnatal day (P) 0), toe-clipped for identification, and were weaned at P21. Prenatal treatments did not significantly alter pregnancy length (22 days) and a Chi square test showed that there were no significant differences in litter size among the treatment groups ($\chi^2(15) = 8.15$, $p > .05$, $M = 13.06$ pups, $SD = 2.67$). On P21, pups were weaned and culled to 8 or 10 animals per litter. Weaned rats were housed in same-sex, same-drug groups of 2–3, in standard polypropylene cages under the same conditions as dams, unless otherwise stated. All behavioral testing took place during the light phase. Body weight was monitored weekly. All procedures