Behavioural Brain Research 278 (2015) 244–256

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Sexually dimorphic effects of prenatal exposure to lipopolysaccharide, and prenatal and postnatal exposure to propionic acid, on acoustic startle response and prepulse inhibition in adolescent rats: Relevance to autism spectrum disorders

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HIGHLIGHTS

- Metabolic byproducts of gut bacteria may alter brain and behavior of rodents.
- Prenatal PPA and LPS treatment effects on rat startle behavior were examined.
- Prenatal LPS was found to increase the acoustic startle response in male rats.
- Pre- and postnatal PPA was found to decreased prepulse inhibition in females.
- Sex differences in the effects of pre- and postnatal PPA were found in adolescent rats.

ARTICLE INFO

Article history:
Received 27 May 2014
Received in revised form
16 September 2014
Accepted 20 September 2014
Available online 7 October 2014

Keywords:
Neurodevelopmental disorders
Sex differences
Maternal immune activation
Short chain fatty acid
Startle response
Pre-pulse inhibition

ABSTRACT

Potential environmental risk factors for autism spectrum disorders (ASD) include viral/bacterial infection and an altered microbiome composition. The present study investigated whether administration of immune and gastrointestinal factors during gestation and early life altered startle response and prepulse inhibition in adolescent offspring using lipopolysaccharide (LPS), a bacterial mimetic, and propionic acid (PPA), a short chain fatty acid and metabolic product of antibiotic resistant enteric bacteria. Pregnant Long-Evans rats were injected once a day with PPA (500 mg/kg SC) on G12–16, LPS (50 μg/kg SC) on G15 and G16, or vehicle control on G12–16 or G15–16. Male and female offspring were injected with PPA (500 mg/kg SC) or vehicle twice a day, every second day from postnatal days 10–18. Acoustic startle and prepulse inhibition was measured on postnatal days 45, 47, 49, and 51. Prenatal and postnatal treatments altered startle behavior in a sex-specific manner. Prenatal LPS treatment produced hyper-sensitivity to acoustic startle in males, but not females and did not alter prepulse inhibition. Subtle alterations in startle responses that disappeared with repeated trials occurred with prenatal PPA and postnatal PPA treatment in both male and female offspring. Prenatal PPA treatment decreased prepulse inhibition in females, but not males. Lastly, females receiving a double hit of PPA, prenatal and postnatal, showed sensitization to acoustic startle, providing evidence for the double hit hypothesis. The current study supports the hypotheses that immune activation and metabolic products of enteric bacteria may alter development and behavior in ways that resemble sensory abnormalities observed in ASD.

1. Introduction

The gastrointestinal tract (GI) is home to over a trillion commensal bacteria, known as the microbiome, that have a bidirectional relationship with the central nervous system and contribute to normal immune system development and homeostasis in both humans and rodents. GI dysbiosis has been implicated in inflammatory

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http://dx.doi.org/10.1016/j.bbr.2014.09.032
0166-4328/

diseases and neuropsychiatric health [23,80,109]. 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 and stress responses (e.g. [29,47,55]).

Imbalances in the composition of the microbiome and the immune sequelae may also contribute to the development or maintenance of autism spectrum disorders (ASD) in children [26,35,58]. Support for this comes from findings of abnormal levels of bacteria flora, including augmented *Clostridia*, *Bacteroidetes*, and *Desulfovibrio*, in the GI tract of autistic children [40,41,90,122]. As these anaerobic bacteria are antibiotic-resistant, repeated early infections in postnatal life treated with antibiotics may provide an enteric environment that promotes the overgrowth of these bacteria and the propensity for intestinal inflammation and associated neuroimmune and neurohormonal changes [20,40].

Metabolic products of these enteric bacteria include short chain fatty acids (SCFA, from carbohydrate metabolism) [39], which at physiological levels are essential for normal and immune associated functions [1,3,10,27], but at higher levels may alter metabolic and immune function and/or exacerbate ASD related behaviors. In propionic acidemia, a neurodevelopmental metabolic disorder characterized by elevated levels of the SCFA propionic acid (PPA), clinically resembles some aspects of autism [38], and a case study of comorbidity of propionic acidemia and ASD has been presented [2]. As well, elevated fecal levels of SCFA have been found in children with ASD [120]. Our laboratory has proposed that PPA, produced by enteric bacteria, may be a potential environmental factor in the development of ASD. Central administration of PPA in adult male rats has produced a number of brain and behavioral changes including hyperactivity and decreased social behavior consistent with ASD [69–71,106,107,114] and has predictive value in many metabolic alterations in a subset of ASD patients [49].

Immune dysfunction may increase the risk for ASD as alterations in the adaptive and innate cellular immune responses have been observed in children with ASD (see [84] for review). Maternal immune activation (MIA) in rodents has been used to investigate the role of the immune system activation in ASD. An inflammatory response is induced using a variety of agents, including influenza and polyinosinic:polycytidylic acid (poly I:C – a viral mimetic). Lipopolysaccharide (LPS, a bacterial mimetic) is the major component of the cell wall of Gram-negative bacteria and is also a by-product of enteric bacteria metabolism. Offspring of dams treated with these immune agents display behavioral deficits in exploratory behavior and social interaction [12,46,98,104,108].

Two recent reports from our laboratory have shown that prenatal exposure to PPA or LPS can have sexually dimorphic effects on development and behavior [43,44]. Prenatal PPA and LPS induced delays in eye opening and prenatal PPA increased anxiety behavior in an open-field test in both sexes [44]. Prenatal PPA also impaired nest-seeking behavior in both male and female pups, suggesting an impairment of olfactory-mediated neonatal social recognition. In addition, adolescent males from PPA treated mothers approached a novel object more than control rats [43].

Sensory abnormalities are reported in children and adults with autism, and they vary in terms of severity and modalities affected [65,72]. In both self-report and parental report, over 90% of those with autism reported unusual responses to sensory stimuli [22,65]. Both hyper-responding (more than typical) and hypo-responding (below normal response) to taste/smell, tactile, visual, and auditory stimuli have been observed, and there is evidence of abnormal sensory integration [5,14,59,65,96]. Difficulties with habituation to sensory stimuli (decreased responding to repeated stimuli over time) have also been observed [6,86], but are not always present in patients [5,97].

In animal models, the acoustic startle response (ASR) is a commonly used measure of sensory responsiveness. The ASR can be modulated in a number of ways, including with a low intensity prepulse and with habituation. Habituation is the reduction in startle response with repeated presentation of the stimulus. Prepulse inhibition refers to a decrease in acoustic startle response level that occurs when the startle stimulus is preceded 30–500 ms by a non-startling stimulus (prepulse). This inhibition effect is presumed to be due to sensory filtering to allow prepulse processing [61]. In human studies prepulse inhibition deficits have also been examine in ASD and other psychopathologies [63] and the reports that are available for ASD present conflicting results [64,74,85,86,91,126]. Recently Kohl et al. [64] examined startle and prepulse inhibition in high functioning autism and obtained enhanced startle magnitude but no differences in startle habituation or prepulse inhibition. In contrast, two previous studies [74,91] on high functioning ASD patients found evidence for attenuated prepulse inhibition.

ASD present in childhood are more prevalent in males, thus, it is important to conduct animal studies on younger animals and investigate both males and females for possible sex differences. Although more attention has been recently focused on adult sex differences in animal models, the majority of studies still use male adults [13], and information on adolescents is lacking. Also, few studies assess ASR, focusing on other behavioral tests, with prepulse inhibition more frequently included in behavioral test batteries. While repeated treatment with LPS throughout gestation has been shown to decrease prepulse inhibition in adolescent male and female rats [98], there are no reports, to our knowledge, of the effects of prenatal LPS administered at specific gestational time points on ASR or prepulse inhibition in adolescent offspring. Previous MIA research has obtained decreases in prepulse inhibition in adult male and female offspring [8,57], as well as adolescent male offspring [124].

Valproate (VPA), a pharmacological treatment for epilepsy, increases the risk of ASD and shares pharmacological properties with PPA [16,21]. This toxin has been used to develop a rodent ASD model (e.g. [103]), with prenatal administration of VPA producing developmental delay and behavioral deficits [37,99]. Prenatal exposure to VPA has resulted in mixed ASR results in the rodent model. Decreased prepulse inhibition in adult male and female offspring, and adolescent male offspring of VPA treated rat mothers, has been observed [73,103,117].

The present study investigated the effects of prenatal treatment with LPS or PPA, on ASR and prepulse inhibition in adolescent male and female offspring. Additionally, a second 'hit' of PPA, in the second postnatal week, was given to act as an early life insult to mimic postnatal production of SCFA from the developing gut [75,76]. This double hit hypothesis has been proposed for schizophrenia, where genetic predisposition leaves individuals vulnerable to an environmental trigger later in life that results in manifestation of the disorder [9]. The "double hit" approach has also been applied to animal models of immune activation using two environmental insults. Immune activation early in life may confer susceptibility to disease or psychopathology in adulthood [52,112,113,119]. Genetics may also confer susceptibility to prenatal or postnatal environmental insults in ASD, or, more than one insult may be required, as in repeated infections in early life. Immune responses can alter the composition of the microbiome of the gastrointestinal tract [7,11]. It is thus possible that prenatal treatment with LPS or PPA may leave offspring vulnerable to the effects of postnatal PPA exposure.

The present study specifically compared the effects of prenatal exposure to LPS and/or PPA, and postnatal exposure to PPA in adolescent male and female rats. Thus, combined treatments of prenatal LPS with postnatal PPA and prenatal PPA with postnatal PPA were examined. These unique combinations of prenatal and postnatal treatments assessed the presence of exacerbated behavioral effects when compared to single treatments alone.