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Research report

Intracerebroventricular injection of propionic acid, an enteric metabolite implicated in autism, induces social abnormalities that do not differ between seizure-prone (FAST) and seizure-resistant (SLOW) rats

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HIGHLIGHTS

- Examined interactive effects of PPA and FAST models of ASD on rat social behavior.
- PPA induced social abnormalities and astrogliosis, regardless of FAST or SLOW strain.
- FAST rats not treated with PPA did not have social deficits compared to SLOW rats.
- FAST rats were hyperactive and had increased astrogliosis compared to SLOW rats.

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ABSTRACT

Autism is a complex neurodevelopmental disorder that is characterized by social abnormalities. Genetic, dietary and gut-related factors are implicated in autism, however the causal properties of these factors and how they may interact are unclear. Propionic acid (PPA) is a product of gut microbiota and a food preservative. PPA has been linked to autism, and PPA administration to rats is an animal model of the condition. Seizure-prone (FAST) and seizure-resistant (SLOW) rats were initially developed to investigate differential vulnerability to developing epilepsy. However, FAST rats also display autistic-like features, and have been proposed as a genetic model of autism. Here we examined the effects of PPA on social behavior in FAST and SLOW rats. A single intracerebroventricular injection of PPA, or phosphate-buffered saline (PBS), was administered to young-adult male FAST and SLOW rats. Immediately after treatment, rats were placed in same-treatment and same-strain pairs, and underwent social behavior testing. PPA induced social abnormalities in both FAST and SLOW rat strains. While there was no evidence of social impairment in FAST rats that were not treated with PPA, these rats were hyperactive relative to SLOW rats. Post-mortem immunofluorescence analysis of brain tissue indicated that PPA treatment resulted in increased astrogliosis in the corpus callosum and cortex compared to PBS treatment. FAST rats had increased astrogliosis in the cortex compared to SLOW rats. Together these findings support the use of PPA as a rat model of autism, but indicate there are no interactive effects between the PPA and FAST models.

1. Introduction

Autism spectrum disorders (ASD) are a class of neurodevelopmental conditions that affects approximately 1 in 88 individuals [1]. The hallmark clinical features of ASD include social abnormalities, though other cognitive, sensory, and motor deficits are common [2,3]. ASD has a high comorbidity with other neurological conditions including epilepsy and attention deficit hyperactivity disorder

Abbreviations: FAST, seizure prone rats; SLOW, seizure resistant rats; PPA, Propionic acid; ADHD, Attention deficit hyperactivity disorder; SCFA, Short chain fatty acid; ICV, intracerebroventricular; PBS, Phosphate buffered saline; GFAP, Glial fibrillary acid protein; PFA, Paraformaldehyde; ANOVA, Analysis of variance.

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(ADHD; [2,4]). While the causal factors of ASD remain unknown, there is strong evidence for a multi-genetic role in ASD [2]. However, research also indicates that environmental and gut-related factors may be important, and that autism may be a multisystem disorder affecting metabolic, immune and gastrointestinal systems [3,5–8]. As it is difficult to address questions surrounding causality and interactions between genetic and environmental factors in the ASD patient setting, the use of animal models may provide insight into these potential relationships.

Propionic acid (PPA) is a short chain fatty acid (SCFA) that has been implicated as a possible gut-derived environmental factor in ASD [5,9,10]. PPA is produced as a fermentation product by many autism associated gut bacteria, and is also a common preservative in food products that may exacerbate ASD symptoms [9,10]. Moreover, PPA can readily cross both the gut-blood and blood-brain barriers, and can have neuroactive effects similar to those implicated in ASD including intracellular acidification, activation of specific G-coupled receptors, alteration of neurotransmitter release and synthesis, gap junction gating, neuroinflammation, free radical formation, altered lipid profiles, mitochondrial dysfunction, and alteration of gene expression [9–11]. Studies administering intracerebroventricular PPA to rodents report social abnormalities, cognitive impairments, and sensorimotor dysfunction [12–14]. Examination of brain tissue from PPA treated rats has revealed reactive astrogliosis, activated microglia, oxidative stress, glutathione depletion, mitochondrial dysfunction, and alteration of phospholipid/acylcarnitine profiles, all of which are consistent with the findings in ASD patients ([10,12–16], [5]). Taken together, PPA may be involved in clinical ASD, and administration of PPA and related SCFA to rodents represents a valid and useful model to investigate the potential role of environmental and/or gut-related factors in the pathogenesis of the condition.

Autism is often co-morbid with seizure disorders, and they may share similar etiologies [17]. Initially developed to provide insight into epilepsy, seizure-prone (FAST) and seizure-resistant (SLOW) rat strains were produced through selective breeding based on seizure susceptibility [18]. Additionally, co-morbid with their increased susceptibility to epileptogenesis, FAST rats have been reported to display ASD-like features including learning and attention deficits, impulsivity, hyperactivity, delays in social development, repetitive behavior, and numerous physiological abnormalities similar to those in ASD, and have therefore been proposed as a unique genetic model of ASD [17,19,20].

Here we aimed to explore how central exposure to the enteric bacterial metabolite, PPA, would affect social behavior in FAST rats with a genetic predisposition to the condition. FAST and SLOW rats were treated with PPA or PBS-vehicle via intracerebroventricular (ICV) injection. Following treatment, rats underwent social behavior testing and then post-mortem immunofluorescence analysis of brain tissue for reactive astrogliosis. The results demonstrated that PPA treatment in both FAST and SLOW rats strains resulted in social abnormalities, consistent with clinical features seen in ASD, as well as reactive astrogliosis in the corpus callosum and cortex. Of note, FAST rats treated with PBS-vehicle did not show social abnormalities relative to SLOW rats treated with PBS-vehicle, which is not consistent with their use as a model of ASD. However, FAST rats did display baseline hyperactivity, as well as astrogliosis, compared to SLOW rats, which may have implications regarding their use as a model of ADHD and their enhanced seizure susceptibility.

2. Material and methods

2.1. Subjects

The FAST and SLOW rat strains used in this experiment were originally developed at McMaster University, Hamilton, Ontario [18]. The FAST and SLOW rats used in current experiments were offspring from the 43rd and 45th breeding generations, and were bred in the Melbourne Brain Centre animal housing facilities. A total of 50 young-adult male rats were used in this study. Rats were 8 weeks old, weighed 200–250 g, and were naïve to all experimental procedures at the time of surgery. After surgery, rats were individually housed in standard acrylic cages (26 cm × 48 cm × 21 cm), kept under a 12:12 light/dark cycle (lights on 7:00 h) at a controlled temperature (21 ± 1 °C), and had *ad libitum* access to food and water for the duration of the experiment. All procedures were in accordance with Australian Code of Practice for the care and use of animals in scientific purposes by The Florey Animal Ethics Committee (12-077-UM).

2.2. ICV cannula surgery

As previously described [12,13], rats were anaesthetized in a sealed Plexiglas box with 4% isoflurane and 2 L/min oxygen flow. After induction of anesthetic, rats were given a subcutaneous injection of analgesic (carprofen, 5 mg/kg), and were placed in a standard stereotaxic device equipped with a nose cover to maintain gas anesthesia (2% isoflurane and 0.5 L/min oxygen) throughout the surgery. Under sterile conditions, rats were implanted with a 23-gauge cannula in the right lateral ventricle, with the tip of the cannula (PlasticsOne, USA) at the following coordinates with reference to Bregma: anterior/posterior −1.4 mm; medial/lateral 1.8 mm; dorsal/ventral −3.0 mm. Four stainless steel screws were inserted into the skull around the cannula to provide an anchor for dental acrylic that affixed the cannula to the skull. The guide cannula was sealed with a removable plug (Plastics One, USA) before and after injection.

2.3. Pre-treatment open field testing

Following a one-week recovery after the cannula surgery, and one day prior to receiving PPA or PBS treatment, each individual rat was placed in the center of the open field and given a 10 min period to freely explore the open field/social behavior testing apparatus [12,21]. The apparatus consisted of a large circular open-field (90 cm diameter, 40 cm high walls) with sawdust bedding covering the floor of the arena. A CD camera was positioned above the center of the arena and the room was equipped with auto-adjustable operating room light. The camera was connected to a computer for recording and analysis using the *TopScan Behavior Analyzing System* (CleverSys, USA). This software objectively tracks rat behavior and computes quantitative variables. Activity in the open field is commonly used to assess motor function and exploratory behavior in rats. Furthermore, as rats prefer a sheltered environment when placed into a novel setting, the amount of time spent and the number of entries into the middle of the open field, which represents a vulnerable and unshielded environment, is used as an indicator of anxiety-like behavior [22]. As such, the following activity and anxiety-related behavior measures were calculated here [21,23]:

1. Distance travelled (cm) by each individual rat;
2. Time spent in the middle of the arena (66 cm diameter); and
3. Number of entries into the middle of the arena.

2.4. Experimental groups and ICV injections

Following a one-week recovery from cannula surgery and pre-treatment open field testing, rats were randomly assigned to one of four experimental groups: FAST + PPA (4 μL, 0.26 M; $n = 12$), SLOW + PPA ($n = 14$), FAST + phosphate buffered saline vehicle (PBS) (4 μL; $n = 12$) and SLOW + PBS ($n = 14$). Solutions were buffered to physiological pH 7.5 before injection. Doses were determined from previous dose-response studies [10,12–14].