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Research report

Tactile stimulation improves neuroanatomical pathology but not behavior in rats prenatally exposed to valproic acid

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HIGHLIGHTS

- Prenatal exposure to VPA altered behavior and neuroanatomy of rats.
- Tactile stimulation dramatically reversed VPA-induced changes in neuroanatomy.
- Tactile stimulation early in life acts as a powerful cortical reorganization tool.

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ABSTRACT

Autism is a severe neurodevelopmental disorder with a population prevalence of 1 in 68, and dramatically increasing. While no single pharmacologic intervention has successfully targeted the core symptoms of autism, emerging evidence suggests that postnatal environmental manipulations may offer greater therapeutic efficacy. Massage therapy, or tactile stimulation (TS), early in life has repeatedly been shown to be an effective, low-cost, therapeutic approach in ameliorating the cognitive, social, and emotional symptoms of autism. While early TS treatment attenuates many of the behavioral aberrations among children with autism, the neuroanatomical correlates driving such changes are unknown. The present study assessed the therapeutic effects of early TS treatment on behavior and neuroanatomy using the valproic acid (VPA) rodent model of autism. Rats were prenatally exposed to VPA on gestational day 12.5 and received TS shortly following birth. Whereas TS reversed almost all the VPA-induced alterations in neuroanatomy, it failed to do so behaviorally. The TS VPA animals, when compared to VPA animals, did not exhibit altered or improved behavior in the delayed non-match-to-sample T-maze, Whishaw tray reaching, activity box, or elevated plus maze tasks. Anatomically, however, there were significant increases in dendritic branching and spine density in the medial prefrontal cortex, orbital frontal cortex, and amygdala in VPA animals following early TS treatment, suggesting a complete reversal or remediation of the VPA-induced effects in these regions. The results suggest that postnatal TS, during a critical period in development, acts as a powerful reorganization tool that can ameliorate the neuroanatomical consequences of prenatal VPA exposure.

1. Introduction

Autism is a severe neurodevelopmental disorder that develops in the first 3 years of life. Characterized by impairments in social interactions, communication, and repetitive behaviors, the etiology of autism is not entirely known, but genetic and environmental components have been hypothesized to be involved [1,2]. There is an accumulating body of evidence that *in utero* exposure to valproic acid (VPA), a teratogenic anticonvulsant, leads to an increased risk and incidence of autism [3–5]. In fact, several retrospective human and case studies have documented difficulties in attentional, social, language, and motor abilities among children prenatally exposed to VPA, leading to the idea that valproate exposure during fetal development greatly alters neurodevelopment, including emotional and cognitive functioning [5–12].

In view of the correlation between *in utero* VPA exposure and the incidence of autism in humans – a 4.42% absolute risk [13] – the VPA rodent model of autism was developed [7,9,14]. Prenatal

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exposure to VPA on gestational day 12.5 has proven to be a viable rodent model of autism, as it appears to parallel the anatomical, functional, and behavioral pathology reported in human studies of autism [15]. More specifically, rats prenatally exposed to VPA have been shown to exhibit structural and cellular features in brain similar to those observed in autistic patients, including physical malformations [16], brainstem and cerebellar anomalies [14,17], altered morphology of motor cortex neurons [18], and hyperconnectivity [19]. On a behavioral level, VPA rats have been shown to display many autistic-specific deficits, including decreased social interactions and behaviors, repetitive or stereotypic behaviors, low sensitivity to painful stimuli, and increased anxiety [2,20–22].

In the US alone, autism is estimated to affect 1 in 68 children [22,23,24] and is believed to be on the rise [18]. In fact, rates are considerably higher than those 20 years ago [25]. Given the recent rise in the incidence of autism, the need for remedial and preventative strategies is crucial. Studies aimed at attenuating core autistic behavioral symptoms have primarily undertaken a pharmacological route [26,27]. Although novel pharmacotherapies – such as risperidone and other atypical antipsychotics – have been central in managing related symptoms of autism, treatment of the core symptoms remains a large area of unmet need [28]. As a result, intensive behavioral therapy has recently taken the forefront in numerous interventions targeted at autistic patients [29–36]. In fact, the efficacy of behavioral therapy is related to its positive lifelong implications [37]. For instance, Fields et al. [38] have demonstrated the positive influence of massage therapy among individuals with autism. Given twice a week for 20 min, massage therapy resulted in fewer stereotypical behaviors, reduced touch aversion, and greater social relatedness (in the classroom) among autistic children. Improvements in sleeping patterns, sensory impairments, and social and basic living skills have also been reported following massage therapy [39–41].

Massage therapy involves kinesthetic or sensory stimulation [42], an intervention equivalent to tactile stimulation (TS) in animal studies. Interestingly, animal studies have shown TS to be an effective measure of protection against cortical injury [43,44] and anxiety [45]. TS has also been shown to stimulate maturation in preterm and newborn animals [46,47], and even alter the behavioral and neuroanatomical organization in non-brain injured rats [48]. Given the abundance of literature reinforcing TS as positive enriching experience, it is plausible that such an experience may offer preventative or remedial intervention in animal models of autism.

The purpose of this study was to investigate the magnitude and extent, if any, of the behavioral and neuroanatomical changes induced by prenatal exposure to VPA and whether an early TS treatment can remediate such behavioral and anatomical pathologies. Using a within litter design, half of the rat pups derived from VPA and vehicle-treated dams were given TS treatment. To elucidate the therapeutic effects of TS on behavior in VPA animals, a battery of behavioral assessments were employed: delayed non-match-to-sample T-maze, Whishaw tray reaching task, activity box, and elevated plus maze (EPM). The dendritic organization of the medial prefrontal cortex (Cg3), orbitofrontal cortex (AID), and amygdala was quantified using Golgi methodology.

2. Methods

2.1. Subjects

All experimental protocols were approved in accordance with the Canadian Council of Animal Care and the University of Lethbridge Animal Care Committee. Animals were born and raised in an accredited animal care facility at the University Lethbridge. Twelve dams and twelve male Long-Evans rats were utilized in this study. The control animals were a subset of a larger study conducted by Richards et al. [48] and the same data was utilized. All procedures among the VPA and control animals – from gestation to adult behavioral testing – were identical and conducted during the same time period, to ensure no significant effect of time or any observable behavioral differences. A single male was paired with a single female in a shoebox cage and mating behaviors were observed for 20 min. If mating behaviors were observed during this time interval, the male remained with the female over the next 24 h. If not, the male was removed. The breeding procedure was repeated the next morning and was continued until all male-female pairs were determined to have mated. Throughout the duration of the pregnancy, female rats were housed in pairs. However, upon the birth of rat pups, each mother was housed individually with her litter. The neonates remained with their mothers until weaning on postnatal day 21 (P21), when they were, consequently, housed with their same-sex siblings. Eighty-two pups were born to 7 VPA dams (46 female, 36 male) and sixty-nine pups to 5 control dams (37 female, 32 male). Behavioral testing of pups commenced on P30. Animals were housed in standard polycarbonate shoebox cages and maintained on a 12 h light/12 h dark diurnal cycle. Food and drinking water were available *ad libitum*, with the exception of food restriction during the duration of the Whishaw tray-reaching task.

2.2. VPA administration

Pregnant dams were administered VPA on G12.5. Three days prior to VPA administration (G9–G11), all dams were given 1.5 g/day of peanut butter. The peanut butter was spoon-fed to each individual rat. On G12.5, half of the dams were given peanut butter mixed with 800 mg/kg of VPA, whereas the remaining control dams received peanut butter alone.

2.3. Tactile stimulation

Tactile stimulation (TS) was performed three times a day (09:00, 13:00, and 16:00) for 15 min intervals. Seven VPA and five control litters were utilized, where equal numbers of male and female rat pups, within each litter, were randomly assigned to the tactile stimulation (TS) and non-tactile stimulation (NTS) groups. TS commenced on P3 and continued until weaning. All animals were transported in their home cage to a testing room for the TS session. Dams were removed from the home cage and placed in a transport cage. The home cage, containing all the rat pups, was placed on a heating pad set to 24 °C. During each session, a partition was used to separate the TS and NTS groups. A Swiffer® duster was used to stimulate the TS group. At the end of each session, mothers were returned to the home cage.

2.4. Behavioral methods

2.4.1. Delayed non-match-to-sample T-maze

Testing on the non-match-to-sample T-maze task occurred between P65 and P75. For 10 consecutive days, ten trials were run per day. The task consisted of 2 trials, separated by a 10 s delay. Trial 1 was a forced trial: one arm was blocked, whereas the other arm was open and contained a food reward. Trial 2 was a choice run: an animal had the choice of entering either arm (both arms were open), but the food reward was located in the arm opposite to that of trial 1. The open arm was randomly assigned and altered between trials. That is, a semi-random schedule was employed (for instance, day 1: RLRRLRLLRL; day 2: LRLLRLRRLR). The number of times the animal entered each arm on trial 2 was recorded and