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Research Report

Increased hippocampal cell density and enhanced spatial memory in the valproic acid rat model of autism

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ABSTRACT

Autism is characterized by behavioral impairments in three main domains: social interaction; language, communication and imaginative play; and the range of interests and activities. However, neuronal processing studies have suggested that hyper-perception, hyper-attention, and enhanced memory, which may lie at the heart of most autistic symptoms. Pregnant Wistar rats were administered by either Valproic Acid (VPA, 500 mg/kg) or Phosphate Buffer Saline (PBS) during fetal neural tube development on embryonic day 12.5. All offspring were subjected to various tests. The present study examined social interaction, repetitive behaviors, nociception and tactile threshold, anxiety as well as spatial memory. Histological analyses of cells in five regions of the hippocampus were done to determine neuronal density in both groups. A single intra-peritoneal injection of VPA to pregnant rats produced severe autistic-like symptoms in the offspring. The results showed significant behavioral impairments such as a lower tendency to initiate social interactions, enhanced stereotyped, repetitive behaviors, increased nociception threshold and anxiety at postnatal day (PND) 30 and PND 60. The Morris water maze learning paradigm revealed enhanced spatial memory at PND 60. Furthermore, histological analysis showed that the neuronal density in five separate regions of hippocampus (CA1, CA2, CA3, Dentate gyrus and Subiculum) were increased at PND 67. This work suggests that early embryonic exposure to VPA in rats provides a good model for several specific aspects of autism and should help to continue to explore pathophysiological and neuroanatomical hypotheses. This study provides further evidence to support the notion that spatial memory and hippocampal cell density are increased in this animal model of autism.

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1. Introduction

Autism is a behaviorally defined neurodevelopmental disorder consisting of impairment in reciprocal social interactions, deficits in communication, and restrictive and repetitive patterns of behaviors and interests (Frith and Happé, 2005; Buxbaum and Baron-Cohen, 2013; Baron-Cohen, 2004; Baron-Cohen et al., 1985). Although sometimes not diagnosed until school age, Autism Spectrum Disorders (ASDs) develop early in life and are life-long conditions with implications for education, social development, and community adjustment (Allen and Courchesne, 2001). Autism can occur at any point on the IQ continuum. However, IQ is known to act as a strong predictor of outcome. Asperger's syndrome (AS) is a subgroup on the autistic spectrum. Individuals with AS share many of the same symptoms as seen in autism, but with no history of verbal delay which involved in autism and with an IQ in the average range or above (Baron-Cohen, 2004; Baron-Cohen et al., 1985).

The cause(s) of autism remain unknown and an associated neuropathology has not been clearly established. Given the complexity of features seen in autism, it is probable that several brain areas are dysfunctional. Among the brain regions that have been implicated are the cerebellum, brainstem nuclei, amygdala, hippocampal formation, and various cortical areas. Recent studies of human autopsy material have examined autism as a definable systemic disorder involving multiple pre and post-natal factors that may affect brain development and function (Bailey et al., 1998). The brain tissue of subject with autism shows subtle developmental abnormalities, specifically in those areas concerned with language, movement, facial expression and social behaviors (Bauman et al., 1997). People with autism may show enlarged brain size in the first few years of their lives, with altered migration of cortical, amygdalar and cranial nerve motor neurons, as well as cerebellar purkinje cells (Bauman and Kemper, 1985). Cell counts have shown that compared to controls, autistic brains contain smaller neurons with increased cell density in the amygdala, hippocampus, and granular cell layer of the cerebellar vermis (Baron-Cohen et al., 2000).

Abnormalities of the hippocampus and related limbic structures have been hypothesized to be relevant to the pathophysiology of autism because of their role in learning, social functioning, emotion and functions that are typically disturbed in autism (Bauman and Kemper, 1985; Baron-Cohen et al., 2000; 1985; DeLong, 1992; Bachevalier, 1994). One postmortem study reported that patients with autism had small, densely packed neurons in the CA1 through CA4 hippocampal fields and the subiculum as well as reduced complexity and extent of dendritic arborization in the CA1 and CA4 fields (Aylward et al., 1999). However, other histopathological studies have failed to find similar hippocampal abnormalities (Bailey et al., 1998).

Bauman and Kemper (1985) were the first to observe neuropathology of the amygdala and hippocampus in postmortem cases. They reported abnormally small and densely packed cells, particularly in the medial portion of the amygdala, CA1 and subiculum of the hippocampal formation (Bauman and Kemper, 1985; Schumann et al., 2004). However, their findings have not yet been replicated in human studies and have not been showed in animal models of autism. Structural Magnetic Resonance Imaging (MRI) studies of the amygdala and hippocampus of subjects with autism also have provided inconsistent results (Schumann et al., 2004).

In addition to its role in emotional processing and memory (Cohen et al., 1999), the hippocampus is also involved in spatial learning (Raymond et al., 1996) and verbal novelty detection (Balu and Lucki, 2009). Given the difficulties of people with ASD in responding to and processing socio-emotional cues, it has been suggested that its biological basis includes abnormalities in the development and function of limbic structures, including the amygdala and hippocampus (Schumann et al., 2004).

Preclinical and clinical advancement of novel therapies for the treatment of ASD would be greatly augmented by the existence of translational animal models for preclinical testing. As the diagnostic and clinical outcomes of autism are based solely on behavioral outcome measures, neurobehavioral assessments have been a key focus of preclinical animal models (MacFabe et al., 2007).

Valproic acid (VPA) is an antiepileptic drug that is known to be strongly teratogenic. Indeed, VPA has been shown by numerous cases and epidemiological studies (Ardinger et al., 1998; Lindhout et al., 1992) to greatly increase the risk for developmental delays and autism in the exposed embryo. Women under VPA treatment do typically take this drug throughout pregnancy, but the timing for the teratogenic effect of VPA increasing the risk for autism is shown to be around the time of neural tube closure (Moore et al., 2000; Bescoby-Chambers et al., 2001). Previous studies have explored the results of prenatal VPA exposure during the equivalent embryonic stage in rats and found similar gross abnormalities to those in autism, such as a diminished number of cerebellar Purkinje cells and signs of brainstem damage (Rodier et al., 1997, 1996a, 1996b; Markam et al., 2008; Ingram et al., 2000). Behavioral studies further showed that a number of autistic core symptoms, such as impairments in social interactions and higher sensitivity to sensory stimulation are also present in VPA-treated rats (Roullet et al., 2013; Bristot Silvestrin et al., 2013; Sui and Chen, 2012; Markam et al., 2008; Ingram et al., 2000). How prenatal VPA exposure affects the brain in terms of the spatial memory, neuronal density, and micro-anatomical changes in different regions of hippocampus in general is however unknown. So, in the present study, we verified some of the behavioral patterns corresponding to those frequently observed in rat VPA-model of autism such as social interactions, repetitive behaviors, nociception, and anxiety. Then we analyzed spatial memory and its link with hippocampal formation changes. We confirm that prenatally VPA exposed offspring results in neuronal hyper-density within five different regions of hippocampus which has been proposed on the basis of the behavioral and neuroanatomical similarities to human data as an animal model of autism.

2. Results

52.94% of VPA-exposed pups had tail malformations possibly due to a teratogenic effect of VPA; controls did not have