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Acute prenatal exposure to a moderate dose of valproic acid increases social behavior and alters gene expression in rats

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ABSTRACT

Prenatal exposure to moderate doses of valproic acid (VPA) produces brainstem abnormalities, while higher doses of this teratogen elicit social deficits in the rat. In this pilot study, we examined effects of prenatal exposure to a moderate dose of VPA on behavior and on transcriptomic expression in three brain regions that mediate social behavior. Pregnant Long Evans rats were injected with 350 mg/kg VPA or saline on gestational day 13. A modified social interaction test was used to assess social behavior and social preference/avoidance during early and late adolescence and in adulthood. VPA-exposed animals demonstrated more social investigation and play fighting than control animals. Social investigation, play fighting, and contact behavior also differed as a function of age; the frequency of these behaviors increased in late adolescence. Social preference and locomotor activity under social circumstances were unaffected by treatment or age. Thus, a moderate prenatal dose of VPA produces behavioral alterations that are substantially different from the outcomes that occur following exposure to a higher dose. At adulthood, VPA-exposed subjects exhibited transcriptomic abnormalities in three brain regions: anterior amygdala, cerebellar vermis, and orbitofrontal cortex. A common feature among the proteins encoded by the dysregulated genes was their ability to be modulated by acetylation. Analysis of the expression of individual exons also revealed that genes involved in post-translational modification and epigenetic regulation had particular isoforms that were ubiquitously dysregulated across brain regions. The vulnerability of these genes to the epigenetic effects of VPA may highlight potential mechanisms by which prenatal VPA exposure alters the development of social behavior.

1. Introduction

During nervous system development, neurons exhibit periods of vulnerability to teratogens. One period of vulnerability is neuronal birthdate; *i.e.*, the day on which neurons undergo their final mitosis. In the rat, neurons in some of the cranial nerve nuclei are born on gestational day (G) 12 or G13. These include the principal sensory nucleus of the trigeminal nerve and the motor nuclei of the trigeminal, facial, and hypoglossal nerves (Altman and Bayer, 1980a,b,c). Exposure to ethanol on G12 or G13 results in a permanent reduction in the number of neurons in some cranial nerve nuclei (Mooney and Miller, 2007), as well as alterations in social behavior and gene expression (Mooney and Varlinskaya, 2011; Middleton et al., 2012). The pronounced and permanent social deficits seen throughout adolescence and adulthood were most apparent in male offspring (Mooney and Varlinskaya, 2011; Middleton et al., 2012). Specifically, males prenatally exposed to ethanol and tested as early

Abbreviations: aa, amino acid; AA, anterior amygdala; ANCOVA, analysis of covariance; ANOVA, analysis of variance; CREB, cAMP response element binding protein; CREBBP, CREB binding protein; CV, cerebellar vermis; DNA, deoxyribose nucleic acid; G, gestational day; GABA, gamma-aminobutyric acid; HDAC, histone deacetylase; HDACi, histone deacetylase inhibitor; ID, identity; i.p., intraperitoneal; IUTs, intersection/union tests; mg/kg, milligrams per kilogram body weight; mRNA, messenger RNA; OFC, orbitofrontal cortex; P, postnatal day; P. C., principal component; RNA, ribonucleic acid; RTS, Rubinstein–Taybi syndrome; VPA, valproic acid.

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adolescents, late adolescents, or young adults exhibited a significant reduction of social investigation, contact behavior, and play fighting regardless of age. Older adolescent and adult males and females demonstrated social anxiety indexed by transformation of social preference into social avoidance.

Administration of another teratogen, valproic acid (VPA) at a dose of 350 mg/kg, during the same critical period also decreases neuronal number in cranial nerve nuclei (Rodier et al., 1996) but the effect on social behavior, anxiety-like responses under social circumstances, or gene expression is unknown. For this reason we decided to use this dose to explore the effects of prenatal VPA. Many studies examining behavioral outcome after exposure to VPA use a high dose of the drug (500–800 mg/kg). In these models, animals show altered nociception (Schneider et al., 2001; Schneider and Przewlocki, 2005; Schneider et al., 2008), abnormal fear conditioning and increased anxiety (Markram et al., 2008), repetitive, stereotypic-like behaviors (Schneider and Przewlocki, 2005; Markram et al., 2008; Schneider et al., 2008), decreases in social interactions (Schneider and Przewlocki, 2005; Markram et al., 2008; Schneider et al., 2008; Dufour-Rainfray et al., 2010), and alterations in eye-blink conditioning that are similar to the changes seen in humans with ASD (Stanton et al., 2007). Acute prenatal exposure to a high dose of VPA also has anatomical effects; for example, it reduces the number of Purkinje cells in the cerebellum (Ingram et al., 2000), alters the location of serotonergic cells (Kuwagata et al., 2009), decreases serotonin expression in the hippocampus (Stanton et al., 2007), and alters cortical neuronal connectivity (Rinaldi et al., 2008). Chronic prenatal exposure to a moderate dose (300 or 350 mg/kg) alters hippocampal synaptic plasticity (Zhang et al., 2003), increases complexity of apical dendrite branching in motor cortex (Snow et al., 2008), and decreases complexity of dendrite branching in hippocampal neurons (Raymond et al., 1996). But high doses of this drug can also be toxic to the dam and/or cause fetal death (Vorhees, 1987).

VPA has several mechanisms. Its acute effects may be driven by increases of gamma-aminobutyric acid (GABA) concentrations in the brain (Dufour-Rainfray et al., 2010) *via* inhibition of GABA transaminase (Rosenberg, 2007b). VPA can also have more sustained effects that are mediated by manipulation of DNA-processing and changes in gene transcription that result from its ability to act as a histone deacetylase inhibitor (HDACi) (Phiel et al., 2001; Rosenberg, 2007a). Histone acetylation is a global mark and facilitator of gene activity (Brownell and Allis, 1996). Acetylation yields a negative charge, acting to neutralize the positive charge on histones and decrease the interaction of the N-termini of histones with the negatively charged phosphate groups of DNA. As a consequence, the condensed chromatin is transformed into a more relaxed structure, which facilitates transcription. An even more prolonged influence proposed for VPA is through the induction of replication-independent DNA demethylation (Detich et al., 2003). VPA can reset stable DNA methylation patterns in established non-dividing cells and, therefore, can have wide-ranging and long-term effects on all cell types found in the brain and at any period of life (Detich et al., 2003; Rosenberg, 2007a). Equally interesting is the ability of VPA to also affect the level of acetylation of non-histone proteins (Mannaerts et al., 2010).

Brain regions important for social behavior include the orbitofrontal cortex (OFC), the amygdala, and the cerebellar vermis, among others. A lesion made to the OFC or the amygdala can cause deficits in social behavior (Daenen et al., 2002; Diergaarde et al., 2004; Mah et al., 2004; Rudebeck et al., 2007). Cerebellar vermis forms connections with the limbic system (Strick et al., 2009), and abnormalities of the vermis are associated with a number of disorders, including attention-deficit/hyperactivity disorder, schizophrenia, bipolar disorder, depression, anxiety, and autism (DelBello et al., 1999; Ichimiya et al., 2001; Loeber et al., 2001; Kaufmann et al., 2003; Mackie et al., 2007; Picard et al., 2008; Strick et al., 2009).

In the present study, we tested whether acute exposure to a moderate dose of VPA during a critical period of gestation: (1) produces social deficits and increases social anxiety during adolescence and/or adulthood; and (2) alters gene expression in three areas of the brain that are important for normal social behavior. Different forms of social behavior and social preference/avoidance (an index of social anxiety-like behavior) (Varlinskaya and Spear, 2010) were assessed using a modified social interaction test (Varlinskaya et al., 1999, 2001), which allows assessments of different components of social behavior, and of social motivation indexed *via* a coefficient of social preference/avoidance. Transcriptome expression was also evaluated at two levels: as an aggregate measure of each gene's overall expression which encompassed all expressed isoforms, and at the level of individual exons to identify particular differentially spliced isoforms of each gene.

2. Methods

2.1. Animals

Timed-pregnant Long Evans rats (Taconic, Germantown, NY) were injected intraperitoneally (i.p.) with 350 mg/kg VPA (Sigma, St. Louis, MO) or an equivalent volume of saline on gestational day (G) 13. G1 was designated as the first day on which a sperm-positive plug was identified. All procedures were approved by the Committee for Humane Use of Animals at Upstate Medical University and the Institutional Animal Care and Use Committee at the Syracuse Veterans Affairs Medical Center.

Within 24 h of birth (on postnatal day (P) 0), litters were culled to ten, maintaining the ratio of male and female pups at 1:1 as well as possible. Pups were weaned on P21 and subsequently housed in same-sex groups of four littermates on a reverse light-dark cycle (*i.e.*, lights off from 6 am to 6 pm).

2.2. Social behavior study

2.2.1. Modified social interaction test

Animals were tested on P28, P42, or P75 (early and late adolescence and young adulthood, respectively) as described previously (Mooney and Varlinskaya, 2011). One male and one female from each litter were tested at each age, and each animal was only tested once ($n = 10$ per sex/age for saline-exposed animals, $n = 8$ per age for VPA-exposed males, $n = 9$ for VPA-exposed females tested at P28 or P42, $n = 7$ for VPA-exposed females tested at P75).

Testing was conducted under dim light using Plexiglas (Binghamton Plate Glass, Binghamton, NY) test apparatuses. The test apparatus was divided into two compartments of the same size by a clear Plexiglas partition. The partition contained a semi-circular aperture that allowed animals to move between compartments such that only one animal was able to move through the aperture at a time (Varlinskaya et al., 1999, 2001). The box used for adolescent animals was smaller than that used for adult animals (30 cm × 20 cm × 20 cm for adolescents, 45 cm × 30 cm × 20 cm for adults).

On the day prior to testing, each experimental animal spent 30 min alone in the testing apparatus. Allowing the animal to familiarize itself with the apparatus increases the frequency of social interactions in the later test situation (File and Hyde, 1978; File and Seth, 2003). On the test day, experimental animals were marked with indelible ink for later identification, and placed alone into a holding cage for 30 min, another way to increase social interactions. Animals were then placed into the testing apparatus. Five min later, a non-manipulated same-sex, same-age novel rat was also placed