Brain Research Bulletin 108 (2014) 1–9

Contents lists available at ScienceDirect

Brain Research Bulletin

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

Research report

D-Cycloserine ameliorates social alterations that result from prenatal exposure to valproic acid

Kristen A. Wellmann[a], Elena I. Varlinskaya[b,c,d,e], Sandra M. Mooney[a,c,d,e,*]

[a] Department of Pediatrics, University of Maryland, Baltimore, MD 21201, United States
[b] Department of Psychology, Center for Development and Behavioral Neuroscience, Binghamton University, Binghamton, NY 13902, United States
[c] Developmental Exposure Alcohol Research Center, Baltimore, MD 21201, United States
[d] Developmental Exposure Alcohol Research Center, Binghamton, NY 13902, United States
[e] Developmental Exposure Alcohol Research Center, Syracuse, NY 13210, United States

ARTICLE INFO

Article history:
Received 2 May 2014
Received in revised form 15 July 2014
Accepted 6 August 2014
Available online 15 August 2014

Keywords:
Adolescence
Autism
Prenatal
Rat
Social behavior
Ultrasonic vocalization

ABSTRACT

Prenatal exposure to valproic acid (VPA) alters rodent social interactions in a dose-dependent way: exposure to a high dose of VPA (>500 mg/kg) mid-gestation decreases social interactions whereas a moderate dose of VPA (350 mg/kg) increases peer-directed social behavior. The moderate dose also decreases expression of the mRNA for serine in amygdala and orbitofrontal cortex. In this study, we examined whether D-cycloserine could ameliorate VPA-induced alterations in ultrasonic vocalizations (USVs), social interactions, and locomotor activity. Pregnant Sprague Dawley rats were given intraperintoneal injections of VPA (200 mg/kg each) on gestational days 12, 12.5 and 13; controls were injected with saline. Offspring received a subcutaneous injection of saline or D-cycloserine (32 or 64 mg/kg) either acutely (1 h prior to testing) or repeatedly (once per day for four days). Social interactions were assessed during late adolescence, and USVs were recorded concomitantly. Male and female rats that were exposed to VPA demonstrated more locomotor activity than control animals during habituation to the testing chamber. VPA-exposed males showed increased play fighting. D-Cycloserine normalized the VPA-induced increase in play fighting in males and also increased social motivation in females. When the pair contained a VPA-exposed rat, significantly fewer USVs were emitted and 16% of the vocalizations were of a novel waveform. These effects were not seen in pairs containing VPA-exposed animals that were treated with D-cycloserine. Overall, these findings are consistent with data from other laboratories suggesting that D-cycloserine may be a promising pharmacotherapeutic compound for improving social behavior disorders.

1. Introduction

Prenatal exposure to valproic acid (VPA) alters rodent social behavior and brain structure in a dose-dependent manner. Exposure to high doses of VPA (500–800 mg/kg) decreases social interaction (e.g., Schneider, 2008; Schneider and Przewlocki, 2005; Markram et al., 2008; Dufour-Rainfray et al., 2010). In contrast, a single exposure to 350 mg/kg during mid-gestation in the rat accentuates social behavior and alters gene expression (Cohen et al., 2013). Of note, it decreases expression of the mRNA for serine in the amygdala and orbitofrontal cortex.

Serine plays a critical role in the brain. It binds to the co-agonist, or "glycine" site on the N-methyl-D-aspartate (NMDA) receptor. Binding of a co-agonist plus glutamate is necessary for NMDA receptor activation (Johnson and Ascher, 1987), and receptor characteristics such as affinity for glutamate, internalization, and desensitization can be altered by the co-agonist (e.g., Wolosker et al., 2008). Initially thought to originate in astrocytes, it is now known that D-serine can also be produced by neurons (Wolosker and Radzishevsky, 2013).

Disruption at glutamatergic synapses can contribute to abnormal social behavior (e.g., Kumar and Christian, 2009). Reduced

Abbreviations: ASD, autistic spectrum disorder; DCS, D-cycloserine; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders Fourth Edition; G, gestational day; i.p., intraperitoneal; mg/kg, milligrams per kilogram body weight; P, postnatal day; PB, 0.10 M phosphate buffer pH 7.4; USV, ultrasonic vocalization; VPA, valproic acid.

* Corresponding author at: Department of Pediatrics, University of Maryland, Baltimore, Baltimore, MD 21201, United States. Tel.: +1 410 706 2615; fax: +1 410 706 2611.
E-mail addresses: kwellmann@peds.umaryland.edu (K.A. Wellmann), varlinsk@binghamton.edu (E.I. Varlinskaya), smooney@peds.umaryland.edu (S.M. Mooney).

http://dx.doi.org/10.1016/j.brainresbull.2014.08.001
0361-9230/

social behavior is reported in mice that either have less binding at the glycine site on the NMDA receptor (Labrie et al., 2009) or have decreased expression of the NR1 subunit of the NMDA receptor (Halene et al., 2009). The SHANK2 knockout mouse also has alterations in receptor type, spine number, synaptic transmission, and behavior (Schmeisser et al., 2012; Won et al., 2012). Behaviorally, SHANK2 knockouts demonstrate a repetitive behavior, decreased social interactions, increased locomotor activity, and decreased USV production. Treatment with D-cycloserine (DCS), a partial agonist of glutamate and glycine sites of glutamatergic receptors (Sheinin et al., 2001), can normalize both the aberrant function of the NMDA receptors and the social interaction phenotype seen in the SHANK2 knockout animals (Won et al., 2012). DCS has also shown promise for ameliorating social behavior deficits in murine models of autism (e.g., Benson et al., 2013; Jacome et al., 2011; Burket et al., 2013; Deutsch et al., 2011, 2012).

Given that prenatal exposure to VPA alters social behavior and disrupts glutamatergic balance in the brain, we hypothesized that DCS may ameliorate the VPA-induced behavioral deficits. To test this, we examined social behavior and USVs in rats that were exposed prenatally to a moderate dose of VPA, and tested whether DCS can ameliorate alterations associated with prenatal VPA exposure.

2. Materials and methods

2.1. Animals: prenatal exposure

Timed pregnant Sprague Dawley rats were received on gestational day (G) 6 (Harlan, Fredrick, MD, USA). The first day on which a sperm-positive plug was identified was designated G1. Rats were housed at the University of Maryland School of Medicine in an AAALAC accredited facility. Rooms were temperature-controlled (22 °C) and maintained on a 12-h light (07:00 to 19:00)/12-h dark cycle. All procedures were performed with approval of the Institutional Animal Care and Use Committee (IACUC) at the University of Maryland, Baltimore and were in accordance with the guidelines for animal care established by the National Institutes of Health.

Pregnant dams were given three intraperitoneal (i.p.) injections (G12, G12.5, and G13) of 200 mg/kg VPA each (25%, w/v, sodium valproate (152064; MP Biomedicals, Santa Ana, CA) in physiological saline; pH 7.4). Control females received i.p. injections of an equivalent volume of physiological saline. Litters were culled to ten pups within 24 h of birth on postnatal day (P) 0 and left with their dam. As best as possible, the ratio of male and female pups was maintained as 6:4.

2.2. Animals: postnatal drug exposure

On P21, pups were weaned, and male and female offspring were separated and group housed with same-sex littermates (2–3 rats/cage). Prior to social behavior testing (described below), offspring were injected subcutaneously in the nape with DCS or saline. DCS was given at two doses to males; 32 mg/kg or 64 mg/kg, or at 32 mg/kg to females. The doses chosen were based on published data demonstrating that a dose of 32 mg/kg DCS ameliorated social behavior deficits in 4-week-old mice (Deutsch et al., 2011; Jacome et al., 2011). Subjects were either injected once 1 hr prior to testing (single injection) or once per day for four days (multiple injections) prior to testing. To ensure experimental consistency, animals that received multiple injections were administered their final injection 1 hr prior to testing. Only one male and one female animal from each litter was assigned to any given prenatal/postnatal exposure group (n = 8–12 per group).

2.3. Ultrasonic vocalizations

USVs for pairs of animals (one experimental subject and one non-manipulated social partner) were recorded during a modified social interaction test (see below) using an ultrasound microphone (Condenser Microphone 116H; Avisoft Bioacoustics, Berlin, Germany) placed above the testing box. The sampling rate was 187.5 kHz, and data were recorded in a 16-bit format. Data acquisition (Avisoft-RECORDER Version 4.5; Avisoft Bioacoustics) and assessment software (Avisoft SAS Lab Pro; Avisoft Bioacoustics) were used to assess latency to begin vocalizing, as well as the number of 50 kHz (hedonic calls) and 22 kHz (alarm calls) USVs (for review see Portfors, 2007).

Fifty kHz calls were further assessed by categorizing their waveforms as described elsewhere (Wohr et al., 2008). Waveforms were categorized as: simple (a short flat waveform in which the frequency remains constant; audible playback of such USVs sound like short clear notes), frequency modulated (FM call, a wave form in which the frequency modulates either up or down in frequency, or both; during audible playback FM USVs often sound like birdsong or keyboard trills), harmonic (a waveform in which the frequency changes in a step-like or harmonized fashion; rendered audible these waveforms sound like harmonized notes), and a novel "atypical" waveform (in which the waveform is modulated in frequency characteristic of FM or harmonic waveforms but lacks the distinct form/appearance of typical of either FM or harmonic waveforms. Audible playback of these atypical waveforms reveal them to differ from harmonized notes and trilling song characteristic of harmonic and FM USVs, instead atypical waveform sounds are raspy and discordant). Representative sonograms are shown in Fig. 2. The proportion (%) of each waveform was calculated.

2.4. Social interaction test

On one day between P40 and P45, animals underwent testing in a modified social interaction test using a Plexiglas box (30 cm × 20 cm × 20 cm) that was divided into two equally sized compartments by a clear partition with a semicircular hole (7 cm × 5 cm), allowing one animal at a time to move between compartments as described previously (Mooney and Varlinskaya, 2011; Middleton et al., 2012; Varlinskaya et al., 1999). Prior to any social interaction, experimental subjects were marked on the back with a black Sharpie™ and isolated for a total of 30 min (20 min alone in a holding cage + 10 min habituation to the testing apparatus) in the dimly lit testing room. During the 10-min habituation period, subjects were allowed to explore the testing box. Following habituation, a novel non-prenatally exposed and drug-naïve play partner (matched for sex, age, and weight ± 10 g) was introduced, and their social interactions were videotaped for 10 min.

Five measures were scored by an investigator unaware of experimental condition of any animal: play fighting, social investigation, contact behavior, social motivation, and locomotor activity. Play fighting (or social play) was defined as pinning, pouncing or playful nape attack, following and chasing. Social investigation was defined as the sniffing of any part of the body of the partner, and contact behavior was assessed by summing frequencies of crawling over and under the partner and social grooming. Social motivation was determined from the number of crosses between compartments and whether the experimental animal crossed toward or away from the play partner. It was expressed as a coefficient of social preference/avoidance: coefficient (%) = (crossovers to − crossovers from)/(crossovers to + crossovers from). A positive coefficient denotes social preference, while a negative coefficient denotes social avoidance.