Neuroscience Research 98 (2015) 28–34

Contents lists available at ScienceDirect

Neuroscience Research

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

Characterization of *in utero* valproic acid mouse model of autism by local field potential in the hippocampus and the olfactory bulb

Dania Cheaha[a], Sara Bumrungsri[b], Surapong Chatpun[c], Ekkasit Kumarnsit[a,*]

[a] Department of Physiology, Faculty of Science, Prince of Songkla University (PSU), Hat Yai, Songkhla 90112, Thailand
[b] Department of Biology, Faculty of Science, Prince of Songkla University (PSU), Hat Yai, Songkhla 90112, Thailand
[c] Institute of Biomedical Engineering, Faculty of Medicine, Prince of Songkla University (PSU), Hat Yai, Songkhla 90112, Thailand

ARTICLE INFO

Article history:
Received 3 March 2015
Received in revised form 16 April 2015
Accepted 22 April 2015
Available online 2 May 2015

Keywords:
Autism spectrum disorder
Valproic acid
Hippocampus
Novel
Olfactory bulb
Local field potential

ABSTRACT

Valproic acid (VPA) mouse model of autism spectrum disorder (ASD) has been characterized mostly by impaired ultrasonic vocalization, poor sociability and increased repetitive self-grooming behavior. However, its neural signaling remained unknown. This study investigated the local field potentials (LFPs) in the dorsal hippocampal CA1 and the olfactory bulb while animals exploring a novel open field. VPA was administered at gestational day 13. The results demonstrated three core features of ASD in male offspring. However, there was no difference in Y-maze performance and locomotor activity. Analysis of hippocampal LFP power revealed significantly increased slow wave (1–4 Hz) and high gamma (80–140 Hz) oscillations and decreased theta (4–12 Hz) activity in VPA mice. In the olfactory bulb, VPA animals showed greater slow wave (1–4 Hz) and beta (25–40 Hz) activity and lower activity of low gamma (55–80 Hz) wave. Regression analysis revealed positive correlations between hippocampal theta power and locomotor speed for both control and VPA-exposed mice. There was no significant difference between groups for modulation index of theta (4–12 Hz) phase modulated gamma (30–200 Hz) amplitude. These findings characterized VPA mouse model with LFP oscillations that might provide better understanding of neural processing in ASD.

1. Introduction

Low proportion of autism cases have been attributed to a known genetic component. Special attention has been spent in search for specific causes and novel therapies. Early exposure to teratogens during critical periods of pregnancy results in autism (Dufour-Rainfray et al., 2011; Eadie, 2008). Valproic acid (VPA), an anti-epileptic, is well known to cause fetal-valproate syndrome with autism-like symptoms in human (Williams et al., 2001). In animal studies, *in utero* VPA exposure also showed well-replicated phenotypes that mimic the three core symptoms of human autistic characters which include deficits in social and communication skills and restricted/repetitive behaviors (Kim et al., 2011; Roullet et al., 2013; Schneider and Przewlocki, 2004). These findings also demonstrated similar neurochemical, morphological, and behavioral abnormalities to those found in individuals with autism. It means that the central nervous system is affected by VPA at different levels ranging from molecular, synaptic, neural network to behavioral levels.

Consistent findings have demonstrated remarkable similarities of phenotypes in offspring of both human and experimental animals following prenatal VPA exposure (Gandal et al., 2010; Roullet et al., 2013; Schneider and Przewlocki, 2004). Hence, an animal model of autism with prenatal exposure to VPA appeared to be a plausible model to study ASD-related neurobiological processes. Mostly, VPA rodent models have been identified using behavioral and molecular profiles associated with ASD symptoms (Bambini-Junior et al., 2011; Roullet et al., 2010). However, other findings have been searched for surrogate biomarkers that would allow for better understanding of neuronal network in ASD. Particularly, learning and memory processes following *in utero* VPA exposure remained largely unexplored.

Increased intellectual disability has been detected following *in utero* VPA exposure. Significant amount of children exposed to VPA were found to have low mental quotient scores (Shallcross et al., 2011). Consistently, achievement of learning was impaired in a VPA rat model tested with a radial maze and open field experimental paradigm (Narita et al., 2010). These data strongly confirmed that hippocampus-dependent functions including neuronal

* Corresponding author. Tel.: +66 74 288210; fax: +66 74 446680.
E-mail address: kumarnsit.e@gmail.com (E. Kumarnsit).

http://dx.doi.org/10.1016/j.neures.2015.04.006
0168-0102/

networks and intellectual function are clearly affected in ASD. To gain the insights of neurobiology of VPA animal model, a major focus had shifted from behavioral studies to investigations of information processing in the hippocampus. The hippocampus plays critical roles in encoding, storage, and retrieval of memory.

Consequences of prenatal VPA exposure have been consistently evidenced in the hippocampus. Histological studies demonstrated that prenatal VPA affected the hippocampus (Bristot Silvestrin et al., 2013; Edalatmanesh et al., 2013; Sosa-Díaz et al., 2014). Recently, functional biomarkers directly related to abnormal neural dynamics of ASD were identified using electrical brain activity analysis (Gandal et al., 2010). However, the pattern of information processing in the hippocampal network in VPA animal model remained uninvestigated. In particular, encoding signal of novelty exploration from the hippocampus was expected to provide better understanding of the neurobiological correlates regulating behavioral alterations.

In general, rodents identify each other using olfactory sense mainly (Carr et al., 1976). In particular, the olfactory bulb is well known as a neural substrate for social recognition memory. Previously, disruption of the olfactory bulb was found to impair social recognition memory (Pena et al., 2014). In VPA-exposed mice, olfactory deficits have been found (Favre et al., 2013; Moldrich et al., 2013). However, the association between disrupted olfactory function and social impairment remained unclear. Moreover, the hippocampus is capable of using inputs from non-visuospatial resources especially spatial olfactory information to generate spatial representations (Zhang and Manahan-Vaughan, 2013). Therefore, these two brain areas have been proposed to play a role in spatial learning processing particularly in novelty learning processing.

The aim of this study was to characterize LFPs recorded in the dorsal hippocampus (CA1) and the olfactory bulb in VPA-exposed mice while exploring a novel open field environment to identify differential pattern of oscillation within discrete frequency ranges that would reflect neurobiological bases of behavioral aberrations in VPA mouse model of ASD.

2. Materials and methods

2.1. Animals

In utero VPA-exposed offspring were produced using male and female ICR mice (7–8 weeks olds) supplied by the Southern Laboratory Animal Facility of Prince of Songkla University, Songkhla, Thailand. Following the mating of each pairs, pregnancy was determined by the presence of a vaginal plug on embryonic day 0 (E0). The sodium salt of valproic acid (NaVPA, Sigma, USA) was dissolved in 0.9% saline. On the day E13, the treated dams received a single subcutaneous injection of 600 mg/kg NaVPA while control animals (CTR) received saline. The dose and time of administration was chosen based on the previous study demonstrating that this regime elicits autistic-like characters in offspring (Gandal et al., 2010). Then, the dams were housed individually and allowed to raise their litters. Day of birth was assigned as postnatal day zero (PD0). Ten littermates were selected for each dam. Only male mice were selected for the experiment. Approximately 2–3 male pups per each litter from a total 4 litter per group were randomly selected (CTR $n = 11$, VPA $n = 9$). The same sets of animals were used for examination starting from newborn period for developmental milestone test until adult period for LFP recording and behavioral tests. Number of pups used was restricted to achieve the experimental purposes and refined for optimal amount of animal use. The pups were separated from dam at postnatal day (PD) 23. The experimental protocols for care and use of experimental animals described in the present study were approved and guided by the Animals Ethical Committee of the PSU (MOE 0521.11/287).

2.2. Surgery for intracranial electrode implantation

At approximately 4 months after birth, animals were anesthetized with a cocktail of 150 mg/kg ketamine (Calypsol, Gedeon Richter Ltd., Hungary) and 15 mg/kg xylazine (Xylavet, Thai Maji Pharmaceutical Co., Ltd., Thailand) *via* intramuscular injection and mounted in a stereotaxic frame. Local analgesic, lidocaine (Locana, L.B.S. Laboratory Ltd., Part, Thailand) was applied to the exposed tissue of the head. The electrodes were stereotaxically positioned on the left side of the brain to the olfactory bulb (AP: +4.5 mm, ML: ±1 mm, DV: 2 mm) and dorsal hippocampal CA1 (AP: −2.5 mm, ML: ±1.5 mm, DV: 1.5) according to mouse brain atlas (Franklin and Paxinos, 1998). The reference and ground electrodes were placed at midline overlying the cerebellum. Additional holes were drilled for stainless steel anchor screws. Dental acrylic was used to secure all electrodes on the skull. The antibiotic ampicillin (General Drug House Co., Ltd., Thailand) was applied intramuscularly (100 mg/kg) once a day for 3 days to prevent infection.

2.3. Local field potentials (LFPs) data acquisition and analysis

Animals were allowed to recover for at least 2 weeks and individually housed in a single standard home cage (26 cm × 33 cm × 15 cm). LFPs and locomotor activity were obtained while the animal exploring novel open field environment (Fig. 1A). All LFP signals were amplified and then filtered with a low-pass 1 kHz, high-pass 1 Hz and digitized at 4 kHz by a PowerLab 16/35 system (AD Instruments, Australia) with 16-bit A/D, and stored in a PC computer through LabChart 7.3.7 Pro software. Recorded files were overviewed by using visual inspection and only noise-free signals were used for the analysis. 50 Hz notch filtering was applied to remove the noise from power line artifacts. All LFP signals were processed through 1–200 Hz band-pass digital filter (raw filtered signal, Fig. 1B).

2.3.1. Frequency analysis of LFP signals

For spectral power analysis, power spectral density (PSD) was generated by LabChart software using a Hanning window cosine (window size = 0.976 s, overlaps = 0.488 s). Power spectrum were computed separately for the hippocampus and the olfactory bulb. The PSD in each frequency bin was expressed as the percentage of total power (1–200 Hz) (Fig. 1C and D). The average spectral power were constructed in discrete frequency bands indicated for each group and expressed in frequency domain. In this study, power spectrum in the hippocampal LFP was divided into slow wave (1–4 Hz), theta (4–12 Hz) low gamma (30–80 Hz) and high gamma (80–140 Hz). In addition, power spectrum of the olfactory bulb LFP was divided into slow wave (1–4 Hz), theta (4–12 Hz), beta (25–40 Hz), low gamma (55–80 Hz) and high gamma (140–200 Hz).

2.3.2. Phase–amplitude coupling (PAC) analysis of hippocampal LFP signal

The phase–amplitude coupling analysis was conducted with Brainstorm3 software (Tadel et al., 2011). The modulation index (MI) measures the coupling strength between two discrete frequency ranges of interest: a phase-modulating and an amplitude-modulated frequency. In this study, hippocampal theta–gamma PAC was focused. The raw digitized signals were filtered for theta (4–12 Hz) and gamma (30–200 Hz) oscillation. The interplay between these rhythmic oscillations was investigated in term of theta phase modulation of gamma amplitude and expressed as modulation index. The data of each 5-min period were calculated