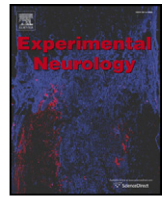
Experimental Neurology 280 (2016) 70–79

Contents lists available at ScienceDirect

Experimental Neurology

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



Hippocampal hyperexcitability in fetal alcohol spectrum disorder: Pathological sharp waves and excitatory/inhibitory synaptic imbalance

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ARTICLE INFO

Article history:
Received 19 September 2015
Received in revised form 29 February 2016
Accepted 13 March 2016
Available online 18 March 2016

Keywords:
Fetal alcohol spectrum disorder
Prenatal alcohol exposure
Electrophysiology
Hippocampus
Hyperexcitability
Sharp waves

ABSTRACT

Prenatal alcohol exposure (PAE) can lead to long-lasting neurological alterations that may predispose individuals to seizures and neurobehavioral dysfunction. To date, there exists limited information regarding the underlying pathophysiological mechanisms. The hippocampal CA3 region generates excitatory population activity, called sharp waves (SPWs), that provide an ideal model to study perturbations in neuronal excitability at the network and cellular levels. In the present study, we utilized a mouse model of PAE and used dual extracellular and whole-cell patch-clamp recordings from CA3 hippocampal pyramidal cells to evaluate the effect of 1st trimester-equivalent ethanol exposure (10% v/v) on SPW activity and excitatory/inhibitory balance. We observed that PAE significantly altered *in vitro* SPW waveforms, with an increased duration and amplitude, when compared to controls. In addition, PAE slices exhibited reduced pharmacological inhibition by the GABA-$_A$ receptor antagonist bicuculline (BMI) on SPW activity, and increased population spike paired-pulse ratios, all indicative of network disinhibition within the PAE hippocampus. Evaluation of PAE CA3 pyramidal cell activity associated with SPWs, revealed increased action potential cell firing, which was accompanied by an imbalance of excitatory/inhibitory synaptic drive, shifted in favor of excitation. Moreover, we observed intrinsic changes in CA3 pyramidal activity in PAE animals, including increased burst firing and instantaneous firing rate. This is the first study to provide evidence for hippocampal dysfunction in the ability to maintain network homeostasis and underlying cellular hyperexcitability in a model of PAE. These circuit and cellular level alterations may contribute to the increased propensity for seizures and neurobehavioral dysfunction observed in patients with a history of PAE.

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1. Introduction

Fetal alcohol spectrum disorder (FASD) is an umbrella term that encompasses all of the physical, physiological and neurobehavioral pathologies induced in offspring as a consequence of *in utero* exposure to alcohol (Sokol et al., 2003). With an estimated prevalence of 2–5% (May et al., 2011, 2014), FASD is the leading preventable cause of intellectual disability in the western world. Epilepsy is another prominent pathology observed in FASD patients that significantly impacts their quality of life. Published studies suggest that, compared to the general population, individuals exposed prenatally to alcohol have a 3 to 20 times increased likelihood of developing seizures (Jones et al., 1973; O'Malley, 1998; Nicita et al., 2014; Bell et al., 2010). Animal models of FASD have also demonstrated reduced pentylenetetrazol-induced seizure threshold, increased susceptibility to spreading depression, and enhanced kindling effects by electrical stimulation (Bonthius et al., 2001a, 2001b).

Several lines of evidence implicate the hippocampus as one of the brain regions responsible for the increased seizure susceptibility observed in individuals with FASD. Firstly, the hippocampus is a well-documented epileptogenic center of the brain (Schwartzkroin, 1994). Secondly, the developing hippocampus is highly sensitive to the teratogenic effects of alcohol, as prenatal alcohol exposure has been shown to induce neuronal cell death (Ikonomidou et al., 2000), and disruption in neuronal migration (Zhou et al., 2001), differentiation (Vallés et al., 1997; Gil-Mohapel et al., 2011), synaptogenesis (Hoff, 1988), and synaptic plasticity (Swartzwelder et al., 1988) within this brain structure.

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http://dx.doi.org/10.1016/j.expneurol.2016.03.013
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Recently Brady et al. (2013) showed in prenatal alcohol-exposed mice that NMDA receptor (NMDAR)-dependent long-term potentiation (LTP) was impaired in the dentate gyrus, which was associated with altered NMDA receptor subunit expression. Conceivably, the documented anatomical and functional deficits induced by prenatal alcohol exposure could lead to long-lasting mis-wiring of hippocampal circuits, predisposing the region to dysfunction and hyperexcitability. To date, whether prenatal alcohol exposure (PAE) restricted to the 1st trimester-equivalent period induces hippocampal hyperexcitability has not been tested.

The specific neuroteratogenic effects of ethanol are highly dependent on the timing, extent, and duration of exposure (Feldman et al., 2012). The first trimester is a time when women may consume alcohol prior to pregnancy recognition, and this period accounts for the great majority of alcohol-exposed pregnancies (Ethen et al., 2009; Colvin et al., 2007). Several animal models have been developed to investigate the effects of ethanol exposure on the 1st trimester of pregnancy. Kaminen-Ahola et al. (2010) developed a mouse model of chronic ethanol exposure that reproduce the craniofacial dysmorphology, postnatal growth restriction, and altered behavior observed in human FASD patients (Sanchez Vega et al., 2013). Importantly, the mice consumed an estimated maximal blood alcohol concentration of 0.12%, which is a realistic measure of moderate ethanol exposure in humans (Jacobson and Jacobson, 1994).

The hippocampus is capable of synchronizing large population of neurons into a multitude of coherent patterns of network activity (Klausberger and Somogyi, 2008; Battaglia et al., 2011). The sharp-wave (SPW) represents an excitatory-dominant population event that forms through slight shifts in the glutamatergic/GABAergic synaptic balance in the CA3 hippocampal area (Ellender et al., 2010; Ylinen et al., 1995; Schönberger et al., 2014; Schlingloff et al., 2014; Hájos et al., 2013). As such, sharp-wave population events are very sensitive to cellular perturbation in activity. For instance, in a disinhibited *in vitro* brain slice preparation, activation of a single pyramidal cell was sufficient enough to entrain SPW events (Menendez de la Prida and Huberfeld, 2006; Miles and Wong, 1983). Notably, the ventral hippocampal slice preparation is also able to spontaneously generate SPWs (Behrens et al., 2005). The facts stated above and the ability to study SPWs within a controlled *in vitro* environment, makes the hippocampal slice preparation an ideal model to assess whether PAE affects network and cellular excitability.

In the present study, using dual extracellular and whole-cell path-clamp recordings obtained from CA3 pyramidal neurons in the ventral hippocampus, we evaluated the effects of 1st trimester-equivalent PAE on SPWs and corresponding cellular activity.

2. Methods

2.1. Animals

We utilized a 1st trimester-equivalent mouse model of voluntary maternal consumption of ethanol (10% v/v) that was adapted from (Kaminen-Ahola et al., 2010). Briefly, 6–8 week old female and male mice (C57BL6, Charles River Laboratories, Montreal, Quebec, Canada) were grouped in the same cage for breeding. Mating was confirmed by the presence of a vaginal plug in female mice. On the day of mating (GD 0.5), the male animal was separated from the cage and the drinking water bottle was replaced with one containing a 10% (v/v) aqueous alcohol solution. Pregnant mice were exposed to 10% (v/v) alcohol by voluntary consumption from GD 0.5 to GD 7.5, which corresponds to the first trimester of gestation in the mouse. The ethanol solution was changed and measured in a period of 24 h. The average daily consumption of ethanol 10% (v/v) was 3.3 mL, or 262.1 mg/day, which was comparable to previous studies (Kaminen-Ahola et al., 2010). Control offspring were obtained from pregnant animals that were not exposed to alcohol during gestation. On PD 15 the body weight of prenatal alcohol exposure (PAE) and control mice was measured. There was a significant decrease in body weight in PAE animal compared to age-matched controls (PAE: 5.8 g; control: 6.5 g, $p = 0.003$, $n = 23$). There was no significant difference between PAE and control groups in average litter size or percentage of animals that delivered live litters. All experiments were conducted on male and female postnatal offspring aged PD 15–21 days that were randomly selected from control and PAE pregnancies. In total, 11 PAE and 17 control litters were used for this study, and from those litters 25 PAE and 46 control animals. The protocols employed in this study conform to the recommendations of the Canadian Council on Animal Care, and this study was approved by the University Health Network animal ethics committee in Toronto.

2.2. Slice preparation

For preparation of hippocampal brain slices, mice were anesthetized with an intra-peritoneal injection of pentobarbital (70 mg/kg body weight) and trans-cardiac infusion with ice-cold oxygenated NMDG (N-methyl-D-glucamine)-based artificial cerebrospinal fluid (aCSF) consisting of (in mM): 92 NMDG, 2.5 KCl, 10 $MgSO_4$, 0.5 $CaCl_2$, 30 $NaHCO_3$, 1.25 NaH_2PO_4, 25 dextorse, 20 HEPES, 2 Thiourea, 5 Na-ascorbate, 3 Na-pyruvate. Subsequently, the brain was rapidly removed, hemisected, and glued onto a block. Transverse slices, 400 μm thick, of the ventral hippocampus were obtained by a vibratome (Leica VT1200) in NMDG-based artificial aCSF oxygenated with 95% oxygen, 5% carbon dioxide (pH 7.4, 280–300 mOsm). Once cut, slices were transferred into an incubation chamber maintained at 34 °C containing aCSF consisting of (in mM): 119 NaCl, 2.5 KCl, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 12.5 dextrose, 2 Thiourea, 5 Na-ascorbate, 3 Na-pyruvate 10 nM kainic acid oxygenated with 95% oxygen, 5% carbon dioxide (pH 7.4, 280–300 mOsm). After 30 min at 34 °C the incubation chamber was set to room temperature. After a 60 min equilibration period, slices were transferred to a submerged recording chamber with a dual perfusion system (Hájos et al., 2013) and perfused at a rate of 15 mL/min with oxygenated aCSF and maintained at 34 °C consisting of (in mM): 123 NaCl, 4 KCl, 1 $MgSO_4$, 1 $CaCl_2$, 25 $NaHCO_3$, 1.2 NaH_2PO_4, 10 dextrose, 10 nM kainic acid oxygenated with 95% oxygen, 5% carbon dioxide (pH 7.4, 280–300 mOsm). Kainic acid at this very low concentration was used to activate intrinsic oscillatory activity through activation of physiological receptors (Florez et al., 2015; Juuri et al., 2010; Tsintsadze et al., 2015).

2.3. Electrophysiological recordings

For extracellular recordings, glass microelectrodes (2–3 MΩ, 1.5 mm outside diameter, World Precision Instruments) were filled with aCSF and placed into the CA3 stratum pyramidale region. Stable spontaneous SPW activity could regularly be recorded from slices after 15–30 min of equilibration. Slices not exhibiting SPW after 1 h were discarded. The mossy fiber tract was stimulated with a bipolar tungsten electrode (enamel-insulated nichrome wire, 125 μM diameter) positioned in the hilus of the dentate gyrus. Extracellular stimulation was generated by a Grass S88 stimulator (Grass Instruments) delivered through an isolation unit. Field EPSP (fEPSP) and population spike (PS) input/output curves were generated by constant current pulses (0.1 ms duration, 1 Hz) ranging from 0.1–1.5 mA. For paired-pulse ratio of fEPSP and PS at different inter-stimulus intervals, stimulation intensity was set to 50% of the maximal response. CA3 pyramidal neurons were visualized by infrared differential interference contrast video microscopy (Olympus BX51 microscope; OLY-150IR camera-video monitor unit). Whole-cell patch recording electrodes (3–4 MΩ) were filled with solution consisting of (in mM): 135 potassium gluconate, 10 NaCl, 1 $MgCl_2$, 2 NaATP, 0.3 NaGTP, 10 HEPES, 0.2 EGTA. Cells were discarded if the access resistance was >25 MΩ or input resistance <40 MΩ or if either of these parameters changed by >20% during the recording. Spontaneous glutamatergic and GABAergic currents were recorded by voltage-

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