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Prenatal ethanol exposure impairs spatial cognition and synaptic plasticity in female rats

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ABSTRACT

Chronic prenatal ethanol exposure (CPEE) can impair long-term potentiation (LTP) in the male hippocampus. Sexually specific alterations were frequently reported in female animals that had been prenatally exposed to ethanol. This study aimed to examine the effects of CPEE on spatial learning and memory, as well as on hippocampal synaptic plasticity in female adolescent rats. Female offspring were selected from dams that had been exposed to 4 g/kg/day of ethanol throughout the gestational period. Subsequently, performance in the Morris water maze (MWM) was determined, while LTP and depotentiation were measured in the hippocampal CA3-CA1 pathway. In the behavioral test, the escape latencies in both initial and reversal training stages were significantly prolonged. Interestingly, LTP was considerably enhanced while depotentiation was significantly depressed. Our results suggest a critical role of synaptic plasticity balance, which may prominently contribute to the cognitive deficits present in CPEE offspring.

Introduction

Fetal alcohol spectrum disorders (FASDs), caused by maternal alcohol consumption during pregnancy, were first described as fetal alcohol syndrome (FAS) (Jones & Smith, 1973). Since then, it has been reported that these devastating disorders are associated with central nervous system (CNS) malformations, mental retardation (Abel, 1984; Danis, Newton, & Keith, 1981), cognitive impairments, and behavioral dysfunctions that can vary in severity, depending on amount of alcohol consumption, duration, and timing of prenatal alcohol exposure (Aronson, Hagberg, & Gillberg, 1997; Goodlett & Eilers, 1997; Goodlett & Lundahl, 1996; Hausknecht et al., 2005). Recent studies have shown that the brain is the most vulnerable organ to ethanol exposure; therefore, further investigation should be made to discover the mechanism and severity of ethanol-mediated impairment (Momino, Sanseverino, & Schüler-Faccini, 2008; Mooney & Miller, 2007).

CPEE-induced brain injury in offspring can manifest as life-long learning and memory deficits (Choi, Allan, & Cunningham, 2005; Gonzalez-Burgos et al., 2006). Among the deficiencies in a variety of behavioral and cognitive domains of the brain caused by prenatal exposure to ethanol, impairments in rodent spatial learning and memory abilities could be directly related to physiological and biochemical alterations in hippocampal circuitry. Spatial learning and memory deficit induced by ethanol exposure can be directly related to physiological and biochemical changes in hippocampal circuitry (Samudio-Ruiz, Allan, Valenzuela, Perrone-Bizzozero, & Caldwell, 2009; Zink et al., 2011). Studies have shown that the LTP of female offspring is significantly enhanced by CPEE; the potential mechanism of this specific effect might be involved in expression of gonadal hormones (Titterness & Christie, 2012). Although LTP in the hippocampus is well known to underlie learning and memory (Collingridge, Peineau, Howland, & Wang, 2010; Malenka & Nicoll, 1999), the function of long-term depression (LTD) on weakening synaptic strength and maintaining synaptic plasticity balance during the tasks cannot be neglected (Collingridge et al., 2010; Kemp & Manahan-Vaughan, 2007; Nicholls et al., 2008). Although previous studies mainly focused on the effect of potentiation on the neural network under the CPEE condition (Christie et al., 2005; Richardson, Byrnes, Brien, Reynolds, & Dringenberg, 2002; Titterness & Christie, 2012), only a few of those studies reported the behavior changes due to the balance of potentiation and depression.

Recently, several investigations revealed that the brains of both female humans and female rodents are more vulnerable than the brains of males to neurotoxic insult during chronic ethanol

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exposure (Butler, Smith, Self, Braden, & Prendergast, 2008). It has been suggested that there are some female-specific alterations in ethanol-treated female animals. Another study reported that dimorphic properties probably reflected the diverse effects of ethanol exposure on N-methyl-D-aspartate (NMDA) receptor-mediated signaling downstream (Butler et al., 2008). Although the effects of ethanol on interactions between pregnant females and fetuses are complex, ethanol readily crosses the placenta and directly affects developing fetal cells and tissues, including those related to development of the CNS (Weinberg, Sliwowska, Lan, & Hellemans, 2008). Logically, there could be specific alterations in both CNS development and CNS function after long-term prenatal ethanol exposure to females. Moreover, in one of our previous studies, it was found that the LTP of CPEE-male adolescent rats was significantly inhibited, while depotentiation was distinctly enhanced compared to that of control male rats (An, Yang, & Zhang, 2013a). A hypothesis was therefore raised that there was a significantly different modification of CPEE on HFS (high frequency stimulation)-induced LTP and LFS (low frequency stimulation)-induced depotentiation in the female offspring rats. To investigate the adverse effects of CPEE on learning and memory induction and maintenance, we focused on the performance in the MWM test, while an attempt was made to interpret the underlying neural mechanism.

Experimental procedures

Animal preparation

Adult virgin female (200–250 g) Wistar rats were obtained from the Laboratory Animal Center, Academy of Military Medical Science of People's Liberation Army, P.R. China. All animals were group-housed with free access to water and food in an established animal house having a 12-h light/12-h dark cycle and a thermo-regulated environment. Two females were paired with two males for 4–5 days until mating was confirmed by observation of a copulatory plug or the presence of sperm in a vaginal rinse under a microscope. The day that mating was confirmed was recorded as embryonic day 0 (E 0). All experiments were performed according to the protocols approved by the Committee for Animal Care at Nankai University and in accordance with the practices outlined in the NIH Guide for the Care and Use of Laboratory Animals.

Prenatal treatment

The ethanol-exposure model was established in reference to that used in previous studies (Carneiro et al., 2005; Ramachandran et al., 2001). Three groups of pregnant rats were investigated:

1) Ethanol group ($n = 7$), in which female rats were administered ethanol by gavage 7 days before being paired with male rats and throughout the gestational period. Four g/kg body weight ethanol was administered from 20% v/v ethanol solutions in distilled water. Rats had full access to chow and water.
2) Pair-fed group ($n = 7$), in which animals were administered, by gavage, a glucose solution isocalorically equivalent to ethanol-derived calories received by the ethanol group, for the same period of time. The rats had full access to water but received the weight of chow consumed by the corresponding CPEE dam during the previous 24-h period.
3) Control group ($n = 5$), in which female animals had *ad libitum* access to standard rat chow and were administered saline via gavage for the same period of time. The day of birth was identified as postnatal day 0 (PD 0). The entire litter and mother were placed in a large plastic bin with shaved wood chips as bedding.

Five litters of control groups and seven litters of other groups were used in the present study. The female offspring of each litter were randomly culled to 2 or 3 pups on PD 1 and weaned on PD 22. 35-day-old Wistar rats were selected for the present experiment. CPEE offspring rats (EF) were randomly divided into two groups: (1) CPEE female offspring group for the MWM test ($n = 7$), and (2) female offspring group for an electrophysiological experiment ($n = 7$). Pair-fed offspring rats (PF) were also randomly divided into two groups: (1) pair-fed female offspring group for the MWM test ($n = 7$), and (2) pair-fed female offspring for an electrophysiological experiment ($n = 7$). Control offspring rats (CF) were randomly divided into two groups as well: (1) control female offspring group for the MWM test ($n = 7$), and (2) control female offspring for an electrophysiological experiment ($n = 7$). The female offspring used in the present study were selected at all stages of the estrous cycle.

Morris water maze experiment

As previously described with modifications (An, Fu, & Zhang, 2015; An, Li, Yang, & Zhang, 2011, 2012; An & Zhang, 2014a; Han, An, Yang, Si, & Zhang, 2014), the MWM consisted of a 1.5-m diameter circular tank filled with water, which was maintained at 25 ± 1 °C. The tank was divided into four quadrants (I, II, III, and IV), and a 10-cm diameter platform was placed in the center of quadrant III. The rats' movements were monitored by a CCD camera connected to a computer, through which data were collected (SLY-WMS 2.1, Sunny Instruments Co. Ltd., China). The task consisted of three stages: initial training (IT), space-exploring test (SET), and reversal training (RT). In the IT stage, there were two sessions (four trials per session) per day from PD 36 to PD 40. In each trial, the animal was released into the water until it reached and stayed on a platform. If it failed to find the platform within 60 s, the animal was placed on it for 10 s. Escape latency and swimming speed were recorded. On PD 41, one trial test was performed in the SET stage. After removing the platform from the tank, the rat was released from quadrant I and swam freely for 60 s. Quadrant dwell time and platform crossings were measured. On PD 42 and 43, the platform was placed in quadrant I. In the RT stage, the rats' reversal-learning ability was examined. The method used and parameters recorded were the same as those in the IT stage.

In vivo LTP and depotentiation recording

On PD 36, the animals were anesthetized with 30% urethane (1.5 g/kg body weight) by intraperitoneal injection. They were then placed in a stereotaxic frame (SN-3, Narishige, Japan) for surgery and recording as described previously (An, Liu, Yang, & Zhang, 2012; An, Yang, & Zhang, 2013b; An & Zhang, 2014b). A bipolar stimulating electrode was placed in the Schaffer collateral/commissural pathway (AP = −4.0 mm, ML = 2.8 mm), and an ipsilateral recording electrode was positioned in the stratum radiatum area of CA1 (AP = −3.2 mm, ML = 2.0 mm). The optimal depth of the electrode was determined by electrophysiological criteria (Leung, 1980). Test stimuli were delivered every 30 s at an intensity that evoked a response of 50% of its maximum (range 0.3–0.8 mA). After the response stabilized, sampling was made under low-frequency stimulations (0.05 Hz) for 20 min as a baseline. After that, HFS (10 trains of 10 stimuli at 100 Hz with 2-s intertrain interval) was delivered to induce LTP. Field excitatory postsynaptic potentials (fEPSPs) were amplified (×100), filtered at 5–50 kHz, digitized, and collected at 20-kHz sample frequency (Scope Software, PowerLab; AD Instruments, Australia) every 60 s for 60 min. After LTP was recorded, LFS (900 pulses of 1 Hz for 15 min) was delivered, and single-pulse recording resumed every