Imbalanced Synaptic Plasticity Induced Spatial Cognition Impairment in Male Offspring Rats Treated with Chronic Prenatal Ethanol Exposure

Lei An, Zhuo Yang, and Tao Zhang

Background: As chronic prenatal ethanol (EtOH) exposure (CPEE) may cause deficiencies in a variety of behavioral and cognitive functions, the aim of present study is to investigate the effects of CPEE on spatial learning and memory and examine the action of CPEE on synaptic plasticity balance in the hippocampus of adolescent male rats.

Methods: The animal model was produced by EtOH exposure throughout gestational period with 4 g/kg bodyweight, while the male offspring rats were used in the study. Morris water maze (MWM) test was performed, and then, long-term potentiation (LTP) and depotentiation were recorded from Schaffer collaterals to CA1 region in the hippocampus.

Results: It was shown that escape latencies in learning period and re-acquisition period were prolonged in CPEE-treated group compared with that in control group. Furthermore, LTP was drastically inhibited, and depotentiation was distinctly enhanced in CPEE-treated group compared with that in control group.

Conclusions: It is suggested that the balance between cognitive stability and flexibility was broken by the bidirectional effects of long-term synaptic plasticity. In addition, the spatial cognition was attenuated by the alteration of synaptic plasticity balance in CPEE-treated male adolescent rats.

Key Words: EtOH, Cognitive Deficits, Long-Term Potentiation and Depotentiation, Synaptic Balance, Male Adolescent Rats.

Despite the fact that ethanol (EtOH) is one of the oldest pharmacological agents known and its abuse continues to be a major social, economic, and public health problem worldwide, exposure to EtOH in utero can negatively impact the development and maturation of the central nervous system (CNS) of the offspring. It is only now beginning to be understood. Chronic prenatal EtOH exposure (CPEE) may cause deficiencies in a variety of behavioral and cognitive domains of brain (Choi et al., 2005; Gonzalez-Burgos et al., 2006; Momino et al., 2008; Samudio-Ruiz et al., 2009; Slivowska et al., 2010). The disruption of rodent spatial learning and memory abilities may be directly related to physiological and biochemical alterations in hippocampal circuitry (Christie et al., 2005; Richardson et al., 2002).

It is well established that hippocampal area is involved learning and memory. Several studies suggested that changes in synaptic plasticity played a central role in this function (Bliss and Collingridge, 1993; Collingridge et al., 2010; Morris, 2003). Although long-term potentiation (LTP) in hippocampus was well known to underlie learning and memory, long-term depression (LTD) in the hippocampus was regarded as a crucial mechanism related to the acquisition of a comprehensive spatial map (Kemp and Manahan-Vaughan, 2007). Our previous study suggested that NMDAR-dependent LTD was required for behavioral flexibility and might act by weakening previously encoded memory traces when new information was learned (Han et al., 2011). However, existing studies primarily focus on the aversive effect of CPEE on potentiation alone in the neural network (Byrnes et al., 2004; Christie et al., 2005; Richardson et al., 2002; Titterness and Christie, 2012,). Evidently, there is a paucity of data on how potentiation and depression are associated with the behavior changes.

In the present study, we tested the hypothesis that CPEE impaired synaptic plasticity by altering the balance between LTP and depotentiation, which played a key role in cognitive stability and flexibility. To investigate the underlying effect of CPEE on learning and memory induction and maintenance, we established an EtOH-impaired animal model and examined the performance of learning and memory by the Morris water maze (MWM). In addition, LTP and depotentiation from hippocampus Schaffer collaterals to CA1 region were recorded. Furthermore, litter size, birth weight, and bodyweight were measured to evaluate the overall physical status of animals, while both brain and hippocampus weights were estimated to examine the overall CNS status.
MATERIALS AND METHODS

Drug Treatment

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 National Institutes of Health’s Guide for the Care and Use of Laboratory Animals.

The EtOH-impaired Wistar rat model was reference to a previous study (Carneiro et al., 2005). In impaired group (n = 8), female rats were administered by gavage before mating and throughout their gestational period with 4 g/kg body weight EtOH from 20% v/v EtOH solutions in distilled water daily. In control group (n = 8), female rats were administered by gavage an equivalent volume of glucose solution isocalorically substituted for EtOH-derived calories for the same period of time. The day of birth was identified as postnatal day 0 (PD 0).

The litter size was randomly culled to 3 or 4 male pups on PD 1 to assure uniformity of litter size between CPEE and pair-fed groups. They were weaned on PD 22. As there were differences between male and female in the development of CNS (Butler et al., 2008; Pfefferbaum et al., 2001; Wang et al., 2003) and synaptic plasticity in particular (Titternness and Christie, 2012), only the male offspring was chosen in the first stage of the study. There were 3 groups of animals, and each of them had fourteen 35-day-old male rats. The CPEE group rats were selected from dams that were administered with EtOH throughout their gestational period. The offsprings were randomly divided into 2 groups: (i) CPEE group for MWM test (n = 7); and (ii) CPEE group for LTP and depotentiation experiment (n = 7). The pair-fed group rats were selected from dams administered with EtOH solution. The offspring rats were also randomly divided into 2 groups: (i) pair-fed group for MWM test (n = 7); and (ii) pair-fed group for LTP and depotentiation experiment (n = 7). In control group, rats were selected from dams that were administered with normal saline. The offspring rats were randomly divided into 2 groups as well: (i) control group for MWM test (n = 7); and (ii) control group for LTP and depotentiation experiment (n = 7).

Morris Water Maze Experiment

On PD 36, MWM test was performed to monitor animal’s spatial learning and memory. As previously described with modifications (An et al., 2012a,b; Li et al., 2011; Quan et al., 2011), the MWM consisted of a 1.5-m-diameter circular tank filled with water (45 cm in depth). Black nontoxic ink was added, and the water was maintained at 25 ± 1°C. The tank was divided into 4 equal quadrants (I, II, III, and IV), and a 10-cm-diameter platform was submerged 2 cm below the water surface in the center of quadrant III. The experiment surroundings with moderate light and kept noiseless. Movement of rats in the maze was monitored by a CCD camera connected to a personal computer, through which data were collected and analyzed. The task consisted of 3 consecutive stages: initial training (IT), space exploring test (SET), and re-acquisition training (RT). In IT stage, rats were subjected to 2 sessions of 4 trials per day for 5 consecutive days from PD 36 to PD 40. In each trial, the rats were released into the water individually from 1 of 4 starting points spaced 90° apart around the perimeter of the tank. They were allowed to swim freely until they reached and stayed on the platform. If they failed to locate the platform within 60 seconds, they were placed on it for 10 seconds. The time required to find the platform (escape latency) and the swimming speed were recorded. Subsequent starting points proceeded in a clockwise manner for the ensuing trials. There were intervals lasting approximately 5 minutes among trials and 9 hours between the 2 sessions. For SET stage on PD 41, the platform was removed from the tank. The rats were released individually into water from the starting point of quadrant I and allowed to swim for 60 seconds. Quadrant dwell time, which means the percentage of time spent in the target quadrant (quadrant III), was measured. There was only 1 test session in this phase. On PD 42 to PD 44, the platform was placed in the center of quadrant I, which was opposite to quadrant III. The rats were trained to find the hidden platform in an opposite position, which was used to examine animal’s ability to re-acquisition a learned skill. In RT stage, each rat was given 2 sessions of training. The method used and parameters recorded were the same as those in IT stage.

In Vivo Long-Term Potentiation and Depotentiation Recording

On PD 36, the animals were anesthetized with 30% urethane with a dosage of 4 ml/kg body weight by intraperitoneal injection. Afterward, they were placed in a stereotaxic frame (SN-3; Narishige, Tokyo, Japan) for surgery and recording as described previously (An et al., 2011; Han et al., 2011; Yang et al., 2011). A small hole was drilled in the skull at the site of the recording and stimulating electrodes. A bipolar stimulating electrode was placed in the Schaffer collateral/commisural pathway (4.0 mm posterior to bregma, 2.8 mm left to midline), ipsilateral to the recording electrode. The recording electrode was positioned in the stratum radiatum of CA1 (3.2 mm posterior to bregma, 2.0 mm left to midline). The optimal depth of the electrode was determined by electrophysiological criteria (Leung, 1980). The whole surgery was carried out on the left side of hippocampus. Test stimuli were delivered to the Schaffer collaterals every 30 seconds at an intensity that evoked a response of 50% of its maximum (range 0.3 to 0.8 mA). Once the response stabilized, sampling was made under single-pulse stimulation (stimulus pulse with 0.2 ms, at 0.05 Hz) for 20 minutes as the baseline. After recording the baseline, high-frequency stimulation (10 trains of 10 stimuli at 100 Hz with a 2-second intertrain interval) was delivered to induce LTP. Field excitatory postsynaptic potentials (fEPSPs) were amplified (∗100), filtered at 5 to 5 kHz, digitized, and collected at 20 kHz sample frequency (Scope software, PowerLab; AD Instruments, New South Wales, Australia) every 60 seconds for 60 minutes. After LTP recording, the evoked responses of the last 20 minutes were normalized and used as the baseline of depotentiation. Subsequently, low-frequency stimulation (LFS) (900 pulses of 1 Hz for 15 minutes) was delivered to induce depotentiation. Following LFS, single-pulse recording resumed every 60 seconds for 60 minutes. Initial data measurement was performed in Clampfit 9.0 (Molecular Devices, Sunnyvale, CA).

Data and Statistical Analysis

All data were presented as mean ± SEM. Data for physical findings and electrophysiological recordings were measured using independent-samples t-test. Two-way repeated measures analysis of variance (ANOVA) was applied for analysis of differences among 3 groups during SET stage. One-way ANOVA was performed on the data from single session during IT stage and RT stage for analysis of differences among these 3 groups. Statistical differences were taken when p < 0.05. The analyses were performed using SPSS 16.0 software (SPSS Inc., Chicago, IL).

RESULTS

Physical Findings

There were detrimental intrauterine effects of CPEE on litter size and birth weight. The litter size number of male rat pups from CPEE-treated dam was smaller than that from control group (Fig. 1A, p < 0.05), while there was no
difference between pair-fed offspring and control offspring (Fig. 1A, \( p > 0.05 \)). The birth weight of rat pups in CPEE group was significantly reduced compared with that in control group (Fig. 1B, \( p < 0.01 \)), which persisted into adolescent (Table 1, \( p < 0.01 \)). Nevertheless, there was no difference in bodyweight between pair-fed offspring and control offspring (Table 1, \( p > 0.05 \)). In addition, the brain and hippocampal weights of rats in CPEE group were significantly lower than that in control group (Fig. 1C,D, \( p < 0.05 \)), but no differences were observed between pair-fed offspring and control offspring (Fig. 1C,D, \( p > 0.05 \)).

**Table 1. Measurements of Rats’ Bodyweight in 2 Groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight (g)</th>
<th>( W_{PD,2} )</th>
<th>( W_{PD,35} )</th>
<th>( \Delta W ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.56 ± 0.64</td>
<td>152.13 ± 8.29</td>
<td>1,812.33 ± 5.29%</td>
<td></td>
</tr>
<tr>
<td>Pair-fed</td>
<td>7.39 ± 0.62</td>
<td>148.81 ± 9.43</td>
<td>1,913.67 ± 5.22%</td>
<td></td>
</tr>
<tr>
<td>CPEE</td>
<td>6.02 ± 0.77</td>
<td>116.97 ± 9.25</td>
<td>1,843.02 ± 7.64%*</td>
<td></td>
</tr>
</tbody>
</table>

\( W_{PD\,2} \) and \( W_{PD\,35} \) Separately represent the mean rats’ weight on postnatal day (PD) 2 and PD 35. \( \Delta W \) (%) represents the normalized bodyweight gain of rats from PD 2 to PD 35. Data are presented as mean ± SEM.

**Morris Water Maze Experiment Results**

Spatial learning and memory of CPEE-treated rats were evaluated in the MWM test. During IT stage, the performance of all rats improved with training. The average escape latencies on successive sessions decreased remarkably from session 1 to session 4 (Fig. 2A-left). There was a significant effect of CPEE treatment, in which the escape latencies were significantly prolonged in CPEE group compared with that in both control group and pair-fed group. As shown in Fig. 2A, rats in CPEE group did considerably increase escape latency compared with that in control group (Fig. 2A-left; \( p < 0.01 \) for days 2, 3 and 4; \( p < 0.05 \) for day 5). There were no differences in swimming speed among these 3 groups throughout testing (Fig. 2B-left, \( p > 0.05 \)). In SET stage, there was significant difference in platform crossings between CPEE group and control group (Fig. 2C, \( p < 0.05 \)). Moreover, the quadrant dwell time was significantly decreased in CPEE group compared with that in control group (Fig. 2D, \( p < 0.01 \)). On the other hand, there were no statistical differences in the above behavior indexes between pair-fed group and control group (Fig. 2A-left, 2B-left, 2C, and 2D, \( p > 0.05 \)). Learning flexibility was then examined on the RT stage after the hidden platform was moved to the contralateral quadrant. During RT stage, the mean escape latency was
calculated for each rat on each of 4 training sessions (Fig. 2A-right). There was a significant effect of CPEE treatment, in which the escape latencies were significantly shortened in control group compared with that in CPEE group. As shown in Fig. 2A-right, rats in CPEE group did show significantly prolonged escape latency compared with that in control group (Fig. 2A-right; $p < 0.01$ for day 7; $p < 0.05$ for day 8 and 9). The results showed that CPEE-treated rats were not able to utilize a spatial strategy to learn the position of the platform. In addition, there were no differences in swimming speed among these 3 groups throughout the test (Fig. 2B-right, $p > 0.05$). On the other hand, there were no statistical differences in the above behavior indexes between pair-fed group and control group in the RT stage of the test (Fig. 2A-right, 2B-right, $p > 0.05$).

**DISCUSSION**

Developmental EtOH exposure was known to adversely affect the CNS function of children. Such EtOH-induced CNS damage in both humans and animals was expressed as long-lasting behavioral problems which included body-weight changes, social difficulties, and learning deficits (Riley and McGee, 2005; Sokol et al., 2003). In the present study, offspring exhibited deficits in cognitive...
flexibility. The fact that the escape latencies in learning and re-acquisition periods were significantly prolonged showed that the rats' learning ability and re-acquisition of learned skill were impaired after CPEE treatment. Moreover, the platform crossings and quadrant dwell time were considerably reduced, which suggested that memory ability of CPEE-treated rats was affected. The underlying mechanism of cognitive impairment was elucidated by modulated hippocampal plasticity with different effects, in which LTP was significantly inhibited, while depotentiation was abnormally enhanced.

The present study showed that male pups, obtained from mothers receiving 4 g/kg bodyweight of EtOH during gestation, displayed a significant decrease in overall bodyweight persisted into adulthood. Furthermore, the small litter size in experimental group showed that there was a detrimental effect of EtOH on embryos in utero. It suggested that EtOH played an important role in embryothalinity. There was a significant decrease in the average weight of brain and hippocampus in CPEE group compared with that in control group on PD 35. In line with these data, EtOH exposure induced microcephaly (Guerri et al., 2009), and provoked impairment of learning and memory, as well as long-term and neurobehavioral dysfunctions (Popovic et al., 2006; Wojniak et al., 2006).

Although maternal blood EtOH concentration (BEC) was not measured in the current study, the dosage of CPEE paradigm was conducted as in a previous study. It indicated that the maternal BECs in CPEE group were increased compared with that in control group (Carneiro et al., 2005). Several other studies reported that prenatal EtOH treatment showed a biodistribution in the brain, which was characterized in detail (Choi et al., 2005; Gonzalez-Burgos et al., 2006; Mominno et al., 2008; Samudio-Ruiz et al., 2009; Sliwowska et al., 2010; Tran and Kelly, 2003). With similar methods, another study, in which rats were exposed to 4 g/kg bodyweight daily via oral administration, showed morphological and histochemical changes in the brains of offspring (Barbier et al., 2008; Evrard et al., 2003; Keiver et al., 2005; Titterness and Christie, 2012). It suggested that chronic prenatal oral administration of EtOH increased the BECs and influenced the hippocampus of offspring.

After CPEE treatment, male offspring exhibited cognitive deficits. The prolonged escape latencies in IT stage showed...
that the rats’ learning ability was damaged by CPEE treatment. However, the fact that the swimming speed remained constant throughout tests for each group suggested that motor function was not the underlying determinant for the prolonged latencies. Importantly, in SET stage, both the decreased number of platform crossings and the decreased number of hidden platform crossings showed a misleading status in CPEE-treated rats. Because of the poor performance of spatial reference learning CPEE-treated rats had to search for the hidden platform around the whole pool, which led them to spend less time in the target quadrant. Eventually, deficit learning and memory contributed to the prolonged escape latencies and then resulted in a worse performance in temporal distribution of the target quadrant and the number of hidden platform crossings in SET stage. Not surprisingly, the dramatic alterations of spatial learning and memory were extensively described (Iqbal et al., 2006; Tiwari and Chopra, 2011).

In RT stage, the hidden platform was moved from quadrant III to quadrant I. Re-acquisition of learned skill is the learning of a new response (e.g., movement of the goal target to a new location) in a familiar environment with consistent environmental stimuli across both initial spatial learning (Walsh et al., 2011). In this phase, a kind of purposeful set-shifting was required to both inhibiting the previously reinforced behavioral strategy and acquiring that of the antecedently declined (Chudasama and Robbins, 2003). Similarly, a previous study reported that performance in the re-acquisition MWM task depended on the capacity for cognitive flexibility, which was attributed to simultaneous enhancement of previously acquired strategy and development of new strategy (Labrie et al., 2009). It was found that CPEE-treated offspring failed to adapt and adjust their behavior following a change of the platform position, suggesting there was a lack of flexibility in behavior (Fig. 2). Actually, this progressive improvement of normal rats was attributed to their trial-dependent manners. Due to the poor performance of spatial reference learning, CPEE-treated rats had to search for the hidden platform around the whole pool, which led them to spend more time in the original quadrant. This suggested that the rats’ flexibility of learning and memory was impaired in CPEE group compared with that in control group. This was in agreement with the observation that exposure to EtOH prenatally disrupted reverse learning (Nash et al., 2007; O’Leary-Moore et al., 2006).

Hippocampal LTP was an essential functional indicator on the strength of synaptic connections and held the key to understand how memories were shaped at the cellular level (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999). The fact that fEPSP slopes were significantly reduced in CPEE group suggested that LTP was impaired, which were consistent with the data of learning period. However, the rapid and automatic aspect of context-specific event encoding was not only dependent on the potentiation of synaptic plasticity but also depotentiation of synaptic plasticity in the hippocampus (Morris, 2006). A previous study indicated that although LTP in the hippocampus was well known to underlie learning and memory, and hippocampal LTD was regarded as a crucial mechanism related to the acquisition of a comprehensive spatial map (Kemp and Manahan-Vaughan, 2007). In fact, memory storage was required that those changes occur bidirectionally to eliminate previous information from network and keep an balance between input/output information (Nicholls et al., 2008). Our data suggested that the early information of CPEE-treated rats was greatly filtered due to depotentiation deficits, while the information traces were still not enhanced because of potentiation deficits. In short, the results suggested that CPEE was able to induce the imbalance of synaptic plasticity in offspring, which explicated the crude performance of the MWM tests in CPEE group.

In the present study, although the impairment of glutamatergic transmission affected hippocampal synaptic plasticity (Chefer et al., 2011; Puglia and Valenzuela, 2010; Zink et al., 2011), the actions of EtOH at the γ-aminobutyric acid (GABA) ergic inhibition also contributed to the impairment of synaptic transmission. In particular, it facilitated GABA release onto hippocampal neurons (Hayward et al., 2004; Li et al., 2006; Sanna et al., 2004), which was consistent with current inhibition of CPEE-induced long-term plasticity. In fact, chronic EtOH could damage hippocampal function by interfering the general balance between excitatory and inhibitory systems (McCool, 2011). Furthermore, the study found that there was a significant decrease in the average weight of either brain or hippocampus in CPEE group, suggesting that the hippocampus was not the only brain region influenced by CPEE. A previous report suggested that CPEE affected the cerebellum (Shirpoor et al., 2009), which shaped hippocampal spatial code (Rochefort et al., 2011). Actually, CPEE-induced cognitive deficits in the current study were also indirectly affected by the impairments of other spatial processing regions, such as the lateral/basolateral amygdala (Silverman et al., 2008), cortex (Nagy, 2008), and amygdala (Roberto et al., 2003).

In summary, our results suggest that insufficient LTP and hyperactive depotentiation affect the processing of spatial information and result in the difficulties of finding hidden platform. The impairment of bidirectional synaptic plasticity balance could be explained by the distinct deficits in cognitive abilities so as to affect the performance in MWM tests. These results are consistent with the hypothesis that LTP and LTD are not independent but an entity in modulating efficiency of spatial cognition.

ACKNOWLEDGMENTS

This work was supported by grants from the National Natural Science Foundation of China (31171053, 11232005) and Tianjin research program of application foundation and advanced technology (12JCZDJC22300, 10JCZDJC19100).
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