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Omega-3 fatty acids can reverse the long-term deficits in hippocampal synaptic plasticity caused by prenatal ethanol exposure

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HIGHLIGHTS

- PNEE negatively affects LTP in the dentate gyrus of male but not female animals.
- Enrichment with omega-3 fatty acids reverses the deficits in LTP in male animals.
- Enrichment with omega-3 fatty acids did not influence LTP in ethanol-exposed females.
- Enrichment with omega-3 fatty acids did not influence LTP in control animals.
- Omega-3 fatty acids may be a viable treatment option for alleviating some of the deficits associated with FASD.

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ABSTRACT

Fetal alcohol spectrum disorders result in long-lasting neurological deficits including decreases in synaptic plasticity and deficits in learning and memory. In this study we examined the effects of prenatal ethanol exposure on hippocampal synaptic plasticity in male and female Sprague-Dawley rats. Furthermore, we looked at the capacity for postnatal dietary intervention to rescue deficits in synaptic plasticity. Animals were fed an omega-3 enriched diet from birth until adulthood (PND55–70) and *in vivo* electrophysiology was performed by stimulating the medial perforant path input to the dentate gyrus and recording field excitatory post-synaptic potentials. LTP was induced by administering bursts of five 400 Hz pulses as a theta-patterned train of stimuli (200 ms inter-burst interval). Ethanol-exposed adult males, but not females, exhibited a significant reduction in LTP. This deficit in male animals was completely reversed with an omega-3 enriched diet. These results demonstrate that omega-3 fatty acids can have benefits following prenatal neuropathological insults and may be a viable option for alleviating some of the neurological deficits associated with FASD.

1. Introduction

The consumption of alcohol during pregnancy can significantly damage the developing brain and result in a number of disorders that are grouped under the term fetal alcohol spectrum disorders (FASD). Recent reports have estimated the prevalence of FASD in young school children to be as high as 2–5% [11] and it is thought that FASD is the most common cause of intellectual disability and preventable birth defects [17].

In rodent models of FASD, it has been well documented that hippocampal synaptic plasticity is reduced in adult males following prenatal ethanol exposure (PNEE) [4,19–21]. Few studies have focused on the female brain but those that have, have shown

Abbreviations: ANOVA, analysis of variance; BAC, blood alcohol concentration; CNS, central nervous system; DHA, docosahexaenoic acid; FASD, fetal alcohol spectrum disorders; GD, gestational day; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; PND, postnatal day; PNEE, prenatal ethanol exposure; PUFA, polyunsaturated fatty acid; SEM, standard error of the mean; TBS, theta-burst stimulation.

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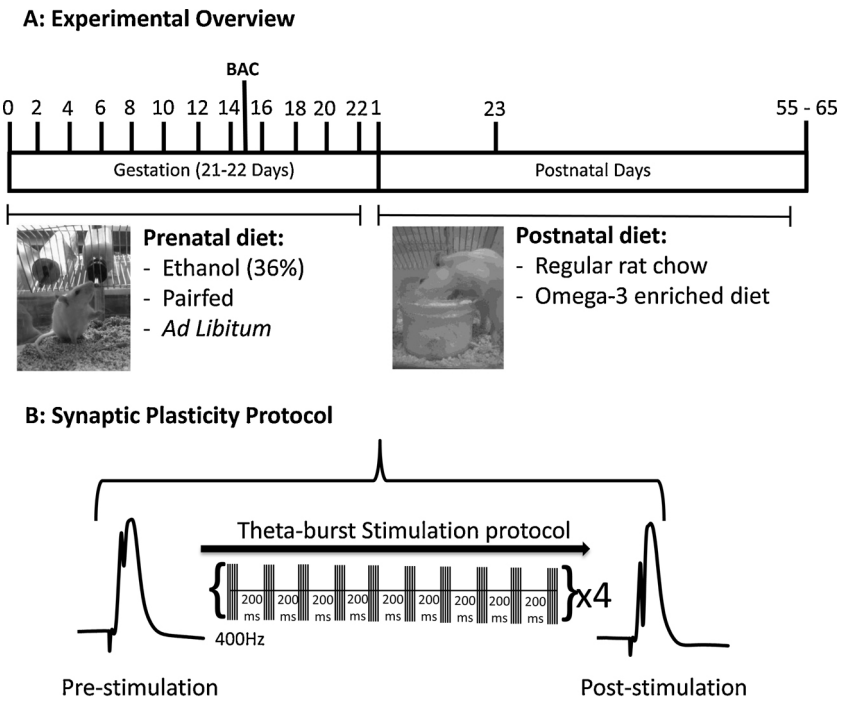

Fig. 1. (A) Experimental Timeline. On GD 1 animals were assigned to one of three prenatal diets (Ethanol, Pair-fed or *Ad libitum* control). On GD 15 a blood sample was taken to assess blood alcohol concentration (BAC). When pups were born the dams were placed on either a regular chow diet or an omega-3 enriched diet. When pups were weaned at PND22 they were continued on the same postnatal diet as their mothers until they reached experimental age (PND55–70). At this point animals were used for *in vivo* electrophysiological experiments. (B) Pre-conditioning evoked responses were obtained by administering a pulse (0.12 ms in duration) at 0.067 Hz (Pre-stimulation). Once a stable baseline was observed for at least 15 min, LTP was induced by applying theta burst stimulation (TBS) consisting of 10 bursts of 5 pulses at 400 Hz with an inter-burst interval of 200 ms, which was repeated 4 times at 30 s intervals. The pulse duration was changed to 0.25 ms during TBS. Following TBS, baseline stimulation was resumed for 60 min at 0.067 Hz (Post Stimulation).

enhanced synaptic plasticity in adolescence [20]. There are very few studies that have tried to rectify the deficits in synaptic plasticity following PNEE [4,21].

Omega-3 fatty acids are polyunsaturated fatty acids (PUFAs) that are found in high concentrations in neuronal membranes [16]. The benefits of omega-3 fatty acid supplementation on synaptic plasticity have been shown in both the dentate gyrus and Cornu Ammonis region of the hippocampus, but only in the aged brain or following some sort of insult (i.e. trauma) [3,8–10,12]. In the healthy brain, omega-3 fatty acid supplementation does not appear to affect LTP, but may prevent age related declines in LTP from occurring [3,10,12]. It is thought that omega-3 supplementation can improve LTP through reducing oxidative stress, inhibiting apoptosis and enhancing membrane fluidity [3,9,10,12,15].

Since PNEE decreases brain concentrations of omega-3 fatty acids [2,22] and omega-3 fatty acid supplementation has been shown to improve synaptic plasticity in other disease or injury models, this study aimed to determine whether feeding an omega-3 fatty acid-enriched diet from birth is able to overcome the deficits in synaptic plasticity that occur with PNEE.

2. Materials and methods

Four male (300–350 g) and 24 virgin female (250–275 g) Sprague-Dawley rats were obtained from Charles River Laboratories (Quebec, Canada). Females were paired with males for breeding purposes and when sperm was detected through vaginal lavage the female was placed onto one of three prenatal diets (*Ad libitum* control, pair-fed or ethanol). The methods for administration and composition of the diets are exactly as found in [14] and are shown in Fig. 1A. A blood sample was taken on GD 15 to determine blood alcohol concentration (BAC). On the final day of pregnancy (GD 21), half of the animals were switched to regular rat chow while the other half were placed on a semi-synthetic diet containing fish oil (kindly supplied by Dr. Shelia Innis, University of British Columbia, Canada) as described in [14]. Litters were culled to ten pups on postnatal day (PND) 2 and were weaned at 22 days of age and housed in pairs (based on sex) in standard caging. Each pup was maintained on either the regular chow diet or the omega-3 enriched diet depending on the diet their mother was assigned to (Fig. 1A). Animals were given *ad libitum* access to the diet from weaning and until they were used for electrophysiological experiments between the age of PND55–70 ($n = 10$ per group).

In vivo electrophysiology procedures were carried out as outlined in [20]. Briefly, a stimulating electrode was placed in the medial perforant path, and a recording electrode was placed into the hilus region of the dentate gyrus. Basal recordings were obtained by administering a pulse (0.12 ms in duration) at 0.067 Hz. Once a stable baseline was observed for at least 15 min, LTP was induced by applying theta burst stimulation (TBS) consisting of 10 bursts of 5 pulses at 400 Hz with an inter-burst interval of 200 ms, which was repeated 4 times at 30 s intervals. The pulse duration was changed to 0.25 ms during TBS. Following TBS, baseline stimulation resumed for 60 min. Excitatory synaptic transmission was characterized through input/output (I/O) function. For analysis of LTP, the slope of the rising phase (10–80%) of the field EPSP at 55–60 min post stimulation was used to determine alterations in the level of synaptic efficacy. All EPSP slope data are presented as the mean percent change from the pre-conditioning baseline. The protocol for these experiments is summarized in Fig. 1B. Females were not used if they were in proestrous, where estrogen levels are highest, as high levels of this hormone can affect LTP and we wanted to reduce biological variation [13,18]. To assess the stage of the estrous cycle, a vaginal lavage with 0.9% sodium chloride was performed each morning and examined with an Olympus Microscope with a 10× objective (Olympus CX21, Center Valley, PA, USA).