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Research report

Prenatal ethanol exposure impairs temporal ordering behaviours in young adult rats

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HIGHLIGHTS

- Hippocampal-dependent behavioral tasks were examined in a model of FASD.
- Prenatal ethanol exposure caused no deficits in a metric change task.
- Prenatal ethanol exposure caused deficits in a temporal order task.
- These deficits were observed in both males and females.

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ABSTRACT

Prenatal ethanol exposure (PNEE) causes significant deficits in functional (i.e., synaptic) plasticity in the dentate gyrus (DG) and *cornu ammonis* (CA) hippocampal sub-regions of young adult male rats. Previous research has shown that in the DG, these deficits are not apparent in age-matched PNEE females. This study aimed to expand these findings and determine if PNEE induces deficits in hippocampal-dependent behaviours in both male and female young adult rats (PND 60). The metric change behavioural test examines DG-dependent deficits by determining whether an animal can detect a metric change between two identical objects. The temporal order behavioural test is thought to rely in part on the CA sub-region of the hippocampus and determines whether an animal will spend more time exploring an object that it has not seen for a larger temporal window as compared to an object that it has seen more recently. Using the liquid diet model of FASD (where 6.6% (v/v) ethanol is provided through a liquid diet consumed *ad libitum* throughout the entire gestation), we found that PNEE causes a significant impairment in the temporal order task, while no deficits in the DG-dependent metric change task were observed. There were no significant differences between males and females for either task. These results indicate that behaviours relying partially on the CA-region may be more affected by PNEE than those that rely on the DG.

1. Introduction

Fetal alcohol spectrum disorders (FASD) is a term used to describe a myriad of neurological deficits and physical abnormalities that can occur when a mother consumes alcohol during pregnancy [1]. The severity and extent of the damage depends on the dosage and frequency of alcohol exposure as well as the genetic and environmental influences of the mother. The most severe form of FASD is fetal alcohol syndrome (FAS), which is characterized by growth deficits, facial dysmorphology, and major cognitive impairments [2,3]. Facial dysmorphology and growth deficits are not often

Abbreviations: ANOVA, analysis of variance; BAC, blood alcohol concentration; CA, *cornu ammonis*; CNS, central nervous system; DG, dentate gyrus; FASD, fetal alcohol spectrum disorders; FAS, fetal alcohol syndrome; GD, gestational day; i.p., intraperitoneal; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; PND, postnatal day; PNEE, prenatal ethanol exposure; S.E.M., standard error of the mean.

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apparent in less severe forms of FASD, but a commonality across the spectrum of disorders is learning and memory impairment [1].

Previous studies from our laboratory [4–7] and others [8–13] have shown that prenatal ethanol exposure (PNEE) causes long-term deficits in synaptic plasticity in the hippocampus. In the *cornu ammonis* (CA) 1 sub-region of the hippocampus, deficits in synaptic plasticity have been observed with prenatal [10,11,13] and postnatal (*i.e.*, 3rd trimester equivalent) [12,14] exposure, but are not always apparent [15–17]. Deficits in LTP in the CA-region appear to occur equally in males and females [10]. PNEE also causes long lasting deficits in synaptic plasticity in the dentate gyrus (DG) sub-region of the hippocampus. In particular, long-term potentiation (LTP) is decreased in this hippocampal sub-field both in adolescent and adult males that were exposed to ethanol prenatally [5–9,18,19]. On the other hand, in females the effects of PNEE on DG LTP appear to be age-dependent, with an increase in DG LTP being detected in adolescent females [7], and no alterations in this form of synaptic plasticity being observed in young adult females [4–6].

The physiological and pharmacological characteristics of LTP, including input specificity, associativity and its long-lasting nature, make it a viable mechanism for learning and memory (for review see Ref. [20]). LTP that requires *N*-methyl-D-aspartate (NMDA) receptor activation exhibits several properties that makes it an attractive candidate of a neurobiological mechanism for memory formation (reviewed in [21]). Blocking NMDA receptors prevents the induction of LTP while also affecting spatial learning and memory as assessed by the Morris water maze test [22]. Several studies have also confirmed that increases in synaptic strength occur in different brain regions, namely the hippocampus, during the processes of learning and memory [23–28].

Deficits in hippocampal-dependent behaviours have been reported in both rodents [10,18,29–36] and humans [37] following PNEE. While in the majority of studies only male animals have been examined, the reports that evaluated both males and females have shown varying results. Thus, while some have reported no sex differences with regards to performance in hippocampal-dependent behavioural tasks [10,34,38–40], others have observed that either males [29–31,41,42] or females [43,44] perform worse in these tasks. The discrepancies in these results are likely due to the different behavioural tests used, the paradigm of alcohol exposure utilized, the blood alcohol concentration (BAC) achieved, and the age of the animals at the time of testing.

To date, hippocampal sub-region-dependent behaviours have not been examined in the context of PNEE. The DG and CA hippocampal sub-regions are believed to be involved in different aspects of spatial information processing, with the DG appearing to be important for pattern separation (*i.e.,* the process of making similar patterns of neural activity distinct from each other), and the CA sub-region playing a role in the processing of the temporal order of spatial patterns or events [45,46]. Goodrich-Hunsaker et al. have developed behavioural tests that examine specific aspects of hippocampal function: a metric change task and a temporal order task, which are believed to rely, at least in part, on the DG and CA1, respectively [47–49].

In the present study we used the metric change (pattern separation) and temporal order behavioural tests to determine whether PNEE differentially affects these hippocampal-dependent behaviours in both males and females during early adulthood.

2. Methods and materials

2.1. Generation of PNEE animals

2.1.1. Animals and breeding

All animal procedures were performed in accordance with the University of Victoria Animal Care Committee and Canadian Council for Animal Care Policies. Four male (300–350 g) and 18 virgin female (250–275 g) Sprague-Dawley rats were obtained from Charles River Laboratories (Quebec, Canada) and housed at the University of Victoria Animal Care Unit. Females were housed in pairs and breeding males were housed individually in clear polycarbonate cages (46 × 24 × 20 cm) with Carefresh contact bedding (Absorption Corp., Bellingham, WA, USA). The room was maintained on a 12-h light:dark cycle with constant humidity and temperature (22 °C). Following an acclimation period for at least one week, females and males were housed together and a vaginal smear using 0.9% sodium chloride (NaCl) was performed at the beginning of each light cycle to determine pregnancy. An Olympus Microscope with a 10× objective (Olympus CX21, Center Valley, PA, USA) was used to detect the presence of sperm. If sperm was detected this indicated gestational day (GD) 1 and the female was immediately removed to a private container supplied with nesting material and placed on one of three prenatal diets (see Section 2.1.2). The liquid diet model of alcohol exposure results in a moderate level of intoxication throughout rodent pregnancy (*i.e.*, during the first and second trimester equivalents). However, since the third trimester equivalent of brain development occurs postnatally in the rat [50], this model does not account for ethanol exposure during this period (for review see Ref. [51]). Despite this, this model presents robust structural and functional neurological deficits, which are comparable with those seen in individuals with FASD [18,52,53].

2.1.2. Prenatal diets

Ethanol-exposed dams had *ad libitum* access to a liquid diet containing 6.6% (v/v) ethanol (i.e., 35.5% ethanol-derived calories) throughout their pregnancy. Ethanol dams were gradually introduced to the liquid diet over a three-day period (GDs 1–3). On GD1, one third of the ethanol liquid diet was combined with two thirds of the pair-fed liquid diet (see below), on GD2, two thirds of the ethanol liquid diet were combined with one third of the pair-fed liquid diet, and on GD3 (and all subsequent days of the pregnancy) only ethanol liquid diet was supplied to the dams. On GD21, all dams was switched back to regular rat chow *ad libitum* (Lab Diets 5001, LabDiets, Richmond, IN, USA).

Pair-fed dams received a liquid diet with maltose-dextrin isocalorically substituted for the ethanol-derived calories. This liquid diet was not provided *ad libitum*. To control for stress and caloric restriction, the pair-fed animals received the same amount of food in g/kg/day as their matched ethanol dams. On GD21, all dams was switched back to regular rat chow *ad libitum* (Lab Diets 5001).

Animals in the *ad libitum* control group received regular chow diet (Lab Diets 5001) *ad libitum* throughout the entire pregnancy.

Liquid diets were obtained from Dyets (Bethlehem, PA, USA) where they are sold as Weinberg/Keiver high protein liquid diet-control (no. 710109) for the pair-fed diet and Weinberg/Keiver high protein liquid diet-experimental (no. 710324) for the ethanol diet. These liquid diets have been nutritionally fortified to ensure that adequate nutrition is provided to the pregnant rats [54]. All liquid