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Research report

Developmental and behavioral consequences of early life maternal separation stress in a mouse model of fetal alcohol spectrum disorder

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HIGHLIGHTS

- 10% alcohol consumption by pregnant mice increases pup novel environment activity.
- Prenatal alcohol exposure leads to hypoactivity in home cage and learning deficits.
- Maternal separation leads to hypoactivity in home environment and learning deficits.
- Behavioural outcomes are regularly influenced by sex.
- Prenatal alcohol exposure and maternal separation stress interactions are modest.

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ABSTRACT

Prenatal alcohol exposure (PAE) can result in fetal alcohol spectrum disorder (FASD), characterized by developmental disability. As children with FASD are often raised in suboptimal conditions, we have investigated the combination of PAE via maternal preference consumption of 10% ethanol in water with early life stress (ELS) via daily 3 h maternal separation and isolation. Our results focus on development and behavioral features, including activity, anxiety-like behavior, as well as learning and memory. PAE influenced the number of pups surviving to postnatal day 2 and 70, with fewer surviving pups associated with the severity of ethanol exposure. PAE and ELS both had effects on pup weight at postnatal day 21, with amount of ethanol exposure positively correlating with pup weight. We found females were more active than males in a novel open field environment, but not following PAE. In addition, PAE resulted in overall increased exploratory behavior in the open field. Further, PAE and ELS both resulted in overnight hypoactivity in a home cage environment, as well as learning deficits that were influenced by sex in the Barnes Maze for learning and memory. These results are attributed to environmental interactions involving PAE and ELS.

1. Introduction

Maternal alcohol consumption during pregnancy may lead to a variety of deficits, collectively termed Fetal Alcohol Spectrum Disorder (FASD) [1]. These deficits include impaired growth, craniofacial abnormalities, and central nervous system dysfunction manifested along a spectrum of severity [2]. Individuals with FASD have poor academic performance, social deficiencies, mental deficits, as well as early and repeated delinquency [3,4]. Despite being entirely biologically preventable, FASD is a serious societal concern and prevalence remains high (1 in 100 live births) in North America [5,6]. Further, many children born with FASD are raised in suboptimal conditions. In Canada, children with a history of PAE represent a significant proportion of children entering child care systems such as foster care or orphanages (6 per 100 children) [7]. Invariably, they face multiple postnatal stresses, including maternal separation. The interaction between PAE and postnatal stress has been explored at different age equivalents, such as adolescence [8] and adulthood [9–11]. The role of early postnatal stresses on the manifestation of FASD-associated behavioral deficits has not been explored to the best of our knowledge. We hypothesize that postnatal stresses associated with maternal separation may compound the deficits in individuals born with FASD. Further, comprehensive

Abbreviations: FASD, Fetal Alcohol Spectrum Disorder; PAE, prenatal alcohol exposure; ELS, early life stress; B6, C57BL/6J mice; CPD, Continuous Preference Drinking Model; FES, ethanol-separated females; MES, ethanol-separated males; FEC, ethanol-control females; MEC, ethanol-control males; FCS, control-separated females; MCS, control-separated males; FCC, control-control females; MCC, control-control males; IV, independent variable; DV, dependent variable.

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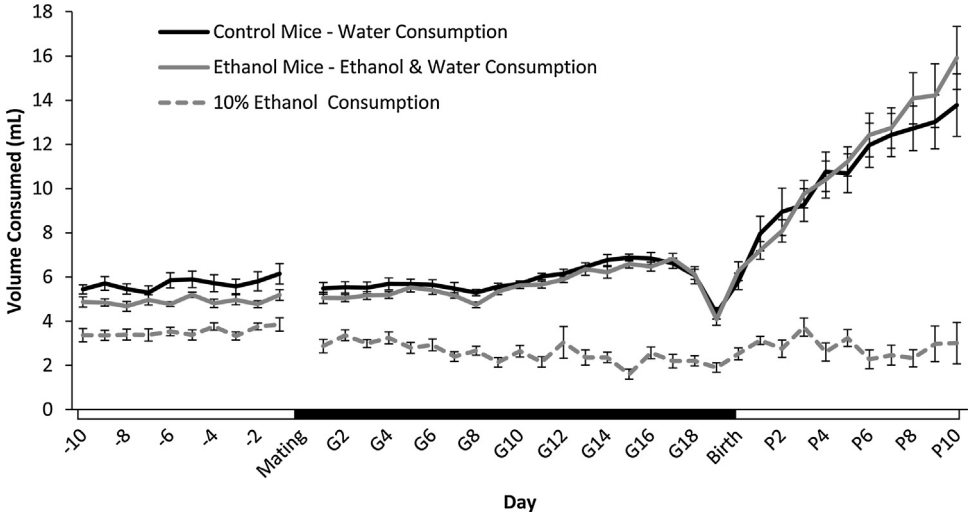

Fig. 1. Mean (±SEM) maternal daily liquid consumption of ethanol-exposed females (Ethanol) and control females (Control) 10 days before mating, during gestation, and 10 postnatal days following birth, N = 13–15.

Table 1
Effect of PAE and ELS on pup survival.

	Ethanol-Separated (ES)	Ethanol-Control (EC)	Control-Separated (CS)	Control–Control (CC)
Total number of pups surviving at P2	32	41	33	43
Total number of pups surviving at P21	30	35	33	42
Weight at P21 (g)	8.22 ± 0.20[a,b]	8.38 ± 0.21[a]	7.29 ± 0.23[b]	8.06 ± 0.26
Total number of pups surviving at P70	30	33	33	41
Percent of pups surviving to P70	94%[c]	80%[c]	100%	95%

[a] Ethanol exposed pups weighed more at P21, $F(1,131) = 5.16$, $p < 0.05$.
[b] Separated pups weighed less at P21 $F(1,131) = 4.28$, $p < 0.05$.
[c] Fewer ethanol exposed pups survived to P70, $X^2(1, N = 149) = 4.75$, $p < 0.05$.

studies on this hypothesis are now feasible with suitable animal models.

C57BL/6J (B6) mice voluntarily consume high, biologically relevant volumes of ethanol when given free access to 10% ethanol in water and water, even while pregnant [12,13]. The pups prenatally exposed to ethanol develop learning deficits, anxiety-like behaviors, and changes in activity [13–16]. The degree of these deficits is variable, and may depend on additional factors. One such factor is early life postnatal stresses (ELS) during development that also affect adult behaviors [17]. In particular, ELS introduced by maternal separation and isolation during early development in mice may lead to increased anxiety-like behaviors in adults [18]. Mouse models adapted from rat models of maternal separation have found the first 10 postnatal days to be the most critical period for stress, likely due to the hippocampal plasticity at this time [19]. From this, mouse models of various forms of separation have focused on postnatal days 1–14 [20,21]. While this time corresponds to the prenatal brain growth spurt in humans that occurs during the third trimester [22], this model best mimics the early life deprivation that may be mirrored in humans. Mice beyond postnatal day 14 are independently mobile, and increasingly less dependent on maternal care, having reached many developmental milestones, such as eating solid food. This research sought to model the period of early life when humans are most dependent on an external source of care. We argue that the postnatal environment has the potential to affect the adult behavior in cases of fetal alcohol exposures, supporting a multifactorial model for FASD that often include postnatal stresses. The most realistic mouse model for behavioral outcomes in FASD should include additional postnatal environment, particularly maternal separation stress often encountered during the development of children born with FASD. To the best of our understanding, such results are not available in the literature, and form the focus of this research. Specifically, we assess the effect of early life maternal separation stress on the development and behavioral outcome in adults resulting from maternal ethanol consumption during pregnancy in mice.

2. Material and methods

2.1. Animals

Male and female B6 mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and subsequently maintained and bred in the Animal Care Facility at Western University. Prior to breeding, mice were housed in same-sex colonies of up to four individuals per colony, with *ad libitum* access to food and water. Cage, bedding, and nest material were standard between cages. Colony rooms were maintained on a 14/10 h light/dark cycle, humidity between 40 and 60%, and ambient temperature 21–24 °C. All protocols complied with ethical standards established by the Canadian Council on Animal Care and were approved by the Animal Use Subcommittee at Western University.

2.2. Continuous preference drinking model

Female mice approximately ten weeks of age were individually housed and randomly assigned to one of two groups: control dams with free access to water only (C), or voluntary ethanol consumption dams with free access to both water plus a 10% ethanol in water solution (E) in a model of alcohol exposure referred to as the Continuous Preference Drinking Model (CPD) [13]. The E group females were introduced to ethanol in a step-wise manner with increasing concentrations of ethanol available from 2%, 5%, and finally 10%, each introduced after 48 h at the previous concen-