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Research report

Behavioral deficits induced by third-trimester equivalent alcohol exposure in male C57BL/6J mice are not associated with reduced adult hippocampal neurogenesis but are still rescued with voluntary exercise

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HIGHLIGHTS

- PD5, 7 and 9 but not PD5 alcohol exposure impairs passive avoidance acquisition.
- PD5, 7 and 9 but not PD5 alcohol exposure impairs rotarod performance.
- Neonatal alcohol exposure does not impact adult neurogenesis levels.

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ABSTRACT

Prenatal alcohol exposure can produce permanent alterations in brain structure and profound behavioral deficits. Mouse models can help discover mechanisms and identify potentially useful interventions. This study examined long-term influences of either a single or repeated alcohol exposure during the third-trimester equivalent on survival of new neurons in the hippocampus, behavioral performance on the Passive avoidance and Rotarod tasks, and the potential role of exercise as a therapeutic intervention. C57BL/6J male mice received either saline or 5 g/kg ethanol split into two *s.c.* injections, two hours apart, on postnatal day (PD)7 (Experiment 1) or on PD5, 7 and 9 (Experiment 2). All mice were weaned on PD21 and received either a running wheel or remained sedentary from PD35-PD80/81. From PD36-45, mice received *i.p.* injections of 50 mg/kg bromodeoxyuridine (BrdU) to label dividing cells. Behavioral testing occurred between PD72-79. Number of surviving BrdU+ cells and immature neurons (doublecortin; DCX+) was measured at PD80-81. Alcohol did not affect number of BrdU+ or DCX+ cells in either experiment. Running significantly increased number of BrdU+ and DCX+ cells in both treatment groups. Alcohol-induced deficits on Rotarod performance and acquisition of the Passive avoidance task (Day 1) were evident only in Experiment 2 and running rescued these deficits. These data suggest neonatal alcohol exposure does not result in long-term impairments in adult hippocampal neurogenesis in the mouse model. Three doses of ethanol were necessary to induce behavioral deficits. Finally, the mechanisms by which exercise ameliorated the neonatal alcohol induced behavioral deficits remain unknown.

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1. Introduction

Approximately 2–5% of all live births in the US are diagnosed as cases of Fetal Alcohol Spectrum Disorders (FASD), an unchanging number despite clear knowledge and the preventable nature of FASD [48]. In humans, prenatal alcohol exposure produces a wide range of long-lasting neurobehavioral deficits, including physical, cognitive, learning and behavioral disabilities [7]. For example, FASD patients exhibit long-lasting deficits in working memory and behavioral flexibility [14,58], behaviors that require the proper functioning of multiple brain regions at once, many of which are still developing during and appear particularly sensitive to alcohol exposure during the third-trimester.

The third trimester is comparable to the first two weeks of postnatal life in mice and rats in terms of brain development [17]. A

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large literature has established that rodents neonatally exposed to alcohol exhibit impaired performance on a variety of behavioral tasks as adults (for review see [54]. Of note, only a few studies have demonstrated a significant influence of neonatal alcohol exposure on Passive avoidance performance [1,3,4,61], a task in which mice must learn and remember to inhibit their natural response to move to the dark side of the chamber and instead stay in the lit side of the chamber. This task involves multiple brain regions [38,69], including, but not limited to, the prefrontal cortex and the hippocampus, two brain regions well-known for being particularly sensitive to third-trimester equivalent alcohol exposure. In fact, Barron and colleagues [3], using an artificial rearing model, demonstrated that third-trimester equivalent alcohol exposure impaired both acquisition and retention of this task in PD23 rats. Whether a similar result is evident in mice and whether this effect persists into adulthood remains unknown. However, given the large number of adult FASD patients suffering from working memory and behavioral flexibility deficits, this is a well-suited behavioral task to use when developing interventions.

Neonatal alcohol exposure produces long-lasting neuroanatomical deficits in the hippocampus of rodents. This is particularly apparent following a third-trimester equivalent exposure [20,46]. Of all the brain regions impacted from alcohol, the hippocampus is arguably the most capable of extensive regeneration and repair [6,47,53]. Still, it is possible that neonatal exposure can produce a persistent reduction in hippocampal plasticity. Some studies show reductions in adult hippocampal neurogenesis and suggest the loss contributes to behavioral deficits [22,27,34,42], while, other studies fail to find an effect of neonatal ethanol exposure on adult neurogenesis [9,22,30,71]. These studies used both rats and mice of different strains. The lack of consensus warrants additional research specifically examining the impact of different timing and duration of alcohol exposures on adult hippocampal neurogenesis in a single mouse strain.

Regardless of whether or not neonatal alcohol exposure results in long-lasting deficits in adult neurogenesis in rodents, exercise is known to increase levels of adult neurogenesis and improve behavioral performance across a broad range of tasks in alcohol-exposed rats [10,21,24,26,60]. Therefore, increasing levels of adult neurogenesis in alcohol-exposed mice could rescue hippocampal function either through compensation or repair. The goal of this study was to determine the extent to which a single (PD7) or repeated (PD5, 7 and 9) binge ethanol exposure reduces adult hippocampal neurogenesis, and impairs behavioral performance on the Rotarod and Passive avoidance tasks, and whether exercise can mitigate any deficits.

2. Materials and methods

Seventy-one (Experiment 1:36; Experiment 2:35) male C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) as adults were used plus an additional 37 pups euthanized at PD7 for blood-ethanol concentration measurements. The 71 mice were generated from 20 pairs (10 pairs per Experiment), and the 37 pups from 7 pairs. The 71 mice were randomized into four groups: saline-sedentary, saline-runner, alcohol-sedentary, alcohol-runner (Experiment 1: n = 9 per group; Experiment 2, n = 7, 8, 10, and 10, respectively). No more than two animals per litter were assigned to a given treatment condition. Mice were housed in standard polycarbonate shoebox cages (29 cm x 19 cm x 13 cm; L x W x H) with corncob bedding (Teklad 7012; Harlan Teklad, Madison, WI, USA). Rooms were controlled for temperature (21 °C ± 1 °C) and photo-period (12:12 D:L; lights off at 10:00am and on at 10:00pm). Food and water was provided *ad libitum*. The Beckman Institute Animal Facility is AAALAC approved. All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee and adhered to NIH guidelines. All efforts were made to minimize the number of mice used and their suffering.

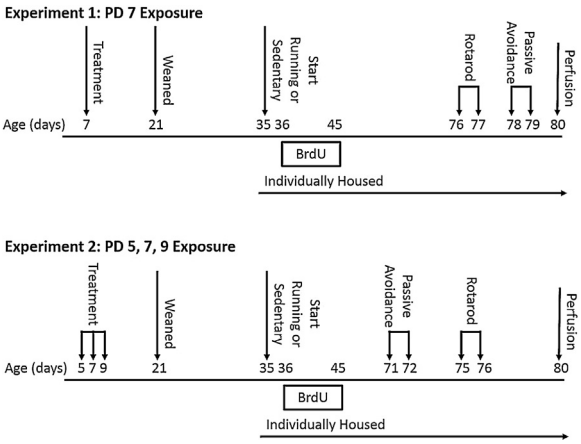

Fig. 1. Experimental Timeline. For Experiment 1, mice were exposed to a single day of treatment, of either sterile saline or 5 g/kg ethanol (split into two doses, two hours apart), on PD 7. For Experiment 2, mice received three days of treatment (sterile saline or 5 g/kg ethanol) on PD 5, 7, and 9. All mice were weaned on PD 21 and singly housed on PD 35, the onset of intervention, which consisted of either voluntary access to a running wheel or sedentary condition. Newly dividing cells were labeled with bromodeoxyuridine (BrdU) for the first ten days of intervention. Mice underwent Passive avoidance and Rotarod testing during adulthood, after being exposed to the assigned intervention for at least thirty days. Differences in the order of the testing procedures were due to the availability of testing equipment.

2.1. Neonatal treatment

Experiment 1: We followed established methods to deliver ethanol to neonatal mice [34,43,71]. Pups from ten litters were injected subcutaneously (*s.c.*) over the shoulders, into the loose skin over the neck, with either a sterile saline solution or a dose of 5 g/kg of ethanol (between 0.03 – 0.08 mL of a 20% ethanol solution per injection; Decon Laboratories, Inc., King of Prussia, PA, USA; 20% ethanol in sterile saline solution; 15.8 mL/kg), which was divided into two injections, two hours apart. To avoid leakage of the solution, the liquid was released slowly into the animal and the needle remained in the animal for 3–5 s following before slowly being removed in order to allow the liquid time to be absorbed. Whole litters were injected with either saline or ethanol. The males from each litter were then assigned to the 2 treatments (runner or sedentary). Litters had 2–4 males, hence there were 1 or 2 males from the same litter per treatment combination. Dose was chosen based on the literature [34,35,43,68,71]. On PD21, all mice were weaned into groups of four by sex (Fig. 1).

Experiment 2: The same procedure as described above was used with the exception that mice received treatments on PD5, 7, and 9.

2.2. Blood ethanol concentration analysis

Trunk blood samples were collected from an additional cohort of 37 mice in order to verify that alcohol concentrations in the blood (BECs) reached a toxic level, above 200 mg/dl for four consecutive hours or more, as BECs at this level have been shown to produce widespread apoptotic neurodegeneration [35]. Mice were assigned to one of six groups at PD7: control (no alcohol administered; n = 3) or alcohol administered then trunk blood collected at the following time points after the first injection of alcohol: 30 min (n = 8), 1 h (n = 8), 3 h (n = 7), 5 h (n = 5), and 9 h (n = 6). Note that for all time points greater than or equal to 3 h, the mice would have received a second injection at 2 h for a total of 5 g/kg. Immediately following collection in heparinized tubes, trunk blood was centrifuged at

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