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Activity and social behavior in a complex environment in rats neonatally exposed to alcohol

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A B S T R A C T

Environmental complexity (EC) is a powerful, stimulating paradigm that engages animals through a variety of sensory and motor pathways. Exposure to EC (30 days) following 12 days of wheel running preserves hippocampal neuroplasticity in male rats neonatally exposed to alcohol during the third-trimester equivalent (binge-like exposure on postnatal days [PD] 4–9). The current experiment investigates the importance of various components of EC (physical activity, exploration, social interaction, novelty) and examines whether neonatal alcohol exposure affects how male rats interact with their environment and other male rats. Male pups were assigned to 1 of 3 neonatal conditions from PD 4–9: suckle control (SC), sham-intubated (SI), or alcohol-exposed (AE, 5.25 g/kg/day). From PD 30–42 animals were housed with 24-h access to a voluntary running wheel. The animals were then placed in EC from PD 42–72 (9 animals/cage, counterbalanced by neonatal condition). During EC, the animals were filmed for five 30-min sessions (PD 42, 48, 56, 64, 68). For the first experiment, the videos were coded for distance traveled in the cage, overall locomotor activity, time spent near other animals, and interaction with toys. For the second experiment, the videos were analyzed for wrestling, mounting, boxing, grooming, sniffing, and crawling over/under. AE animals were found to be less active and exploratory and engaged in fewer mounting behaviors compared to control animals. Results suggest that after exposure to wheel running, AE animals still have deficits in activity and social behaviors while housed in EC compared to control animals with the same experience.

Introduction

Maternal alcohol consumption is a leading cause of preventable mental disability in children (Centers for Disease Control [CDC], 2013). The umbrella term Fetal Alcohol Spectrum Disorders (FASD) includes a wide range of physical, emotional, behavioral, cognitive, and social deficits associated with prenatal alcohol exposure (Clarke & Gibbard, 2003). Despite increased public awareness regarding the dangers of drinking during pregnancy, the current prevalence of FASDs has been estimated to be as high as 5% of live births in the United States (CDC, 2013; May et al., 2009). The influence of FASD is extensive and often results in serious neurodevelopmental deficits affecting cognition and behavior throughout life. Similarly, alcohol exposure has widespread effects on the brain, with the prefrontal cortex, hippocampus, and cerebellum being some of the more vulnerable regions. Developmental alcohol exposure has been shown to lead to decreased gray matter and basal ganglia volume in humans (Mattson et al., 1996; Mattson, Schoenfeld, & Riley, 2001), impaired cortical plasticity (Rema & Ebner, 1999), reduced cortical and hippocampal dendritic complexity and spine density (Hamilton, Akers, et al., 2010; Hamilton, Criss, & Klintsova, unpublished data; Whitcher & Klintsova, 2008), decreased hippocampal adult neurogenesis (Choi, Allan, & Cunningham, 2005; Hamilton, Boschen, Goodlett, Greenough, & Klintsova, 2012; Helfer, Goodlett, Greenough, & Klintsova, 2009; Klintsova et al., 2007), impaired CA1 long-term potentiation (Puglia & Valenzuela, 2010a,b), and reduced cerebellar synapse per Purkinje cell number (Klintsova, Matthews, Goodlett, Napper, & Greenough, 1997). In addition, numerous behavioral deficits are observed, including motor deficits and impaired performance on hippocampal-associated spatial and executive functioning tasks (reviewed in Klintsova, Hamilton, & Boschen, 2013).

Limited pharmacological or behavioral therapies are available for the treatment of children with FASD, though evidence suggests that early interventions result in better outcomes for these children later in life (CDC, 2013). Within the rodent literature, both exercise and exposure to a complex environment have been shown to be beneficial to both the healthy and damaged brain. Housing in classic environmental complexity (EC) paradigms results in increased cortical thickness, enhanced dendritic branching in the frontal,

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temporal, and occipital cortices, and visual cortex synaptogenesis in the normal rat brain (Greenough & Volkmar, 1973; Greenough, Volkmar, & Juraska, 1973; Rosenzweig, Bennett, & Krech, 1964; Turner & Greenough, 1985). Additionally, EC influences various components of cellular neuroplasticity including neurotrophin release, vesicle-docking proteins, and postsynaptic receptor expression (Olson, Eadie, Ernst, & Christie, 2006). These variables may contribute to the beneficial effect of EC on the alcohol-damaged brain. Our lab has demonstrated that a combination of wheel running (WR) followed by environmental complexity enhances hippocampal adult neurogenesis and cortical dendritic complexity in animals exposed to alcohol from PD 4–9 (third-trimester equivalent) (Hamilton et al., 2012; Hamilton, Criss, & Klintsova, unpublished data). In addition, this intervention ameliorates alcohol-associated impairments in contextual fear conditioning, contextual pre-exposure facilitation effect (CPFE) training, and trace eye-blink conditioning (Hamilton et al., 2014; Schreiber et al., 2013).

The design of an effective behavioral intervention for children with FASD is critical for responding to the problem of maternal drinking in our society. The use of rodent models is key in establishing procedures that show the greatest benefit in the least amount of time. The use of a diverse range of EC protocols in the literature makes it difficult to determine which components of the cage contribute most to the beneficial effects. Recent literature on EC paradigms in mice suggests that aerobic exercise is the key factor contributing to the beneficial effects observed (Kobilo et al., 2011; Mustroph et al., 2012). However, EC paradigms that do not include an element that specifically targets increasing aerobic exercise, such as a running wheel, still produce strong neuroplastic changes (Bredy, Humpartzoomian, Cain, & Meaney, 2003; Ehninger & Kempermann, 2003; Fabel et al., 2009; Greenough & Volkmar, 1973; Greenough et al., 1973; Parks, McMechan, Hannigan, & Berman, 2008; Rema & Ebner, 1999; Rosenzweig et al., 1964; Schapiro, 2002; Turner & Greenough, 1985). Additionally, our lab uses an intervention that includes wheel running immediately prior to, but separate from, the EC paradigm, and we have found that EC is able to support long-lasting neuroplastic alterations that may have been initiated during WR.

The current experiment seeks to further understand the influence of various components of EC on animal behavior and whether neonatal alcohol exposure affects how the rats interact with their environment and other animals. Four factors involved in our EC paradigm were considered: 1) physical activity, 2) exploratory behavior, 3) novel enrichment items, and 4) social interactions. The first experiment investigated locomotor and exploratory behavior of rats during 4 weeks in EC by assessing the 4 different factors of the intervention listed above. The second experiment examined the specific social interactions between individual animals while in the EC cage.

Materials and methods

Animals

Rats were bred at the University of Delaware's Office of Laboratory Animal Medicine Facility. On PD 3, 8 litters were culled to 8 pups each (6 male, 2 female when possible). The timeline of the experiment is displayed in Fig. 1A. On PD 4, pups were randomly assigned to 1 of 3 experimental groups: suckle control (SC), sham-intubated (SI), and alcohol-exposed (AE). A split-litter design was used so that SI and AE animals were represented in the same litter. Pups remained with the dam and littermates until weaning on PD 23. Following weaning, rats were housed in groups of 3 same-sex animals in standard cages (17 cm high × 145 cm long × 24 cm wide) until PD 30 (counterbalanced for litter and neonatal condition), when they were placed either into wheel running followed by environmental complexity (described in *Environmental Complexity* section) or maintained in social housing (animals used for other studies reported elsewhere). In total, 18 male rats were used for the current study, 6 per neonatal condition (AE, SI, SC). The animals were housed in a 12/12-h light/dark cycle (lights on at 9:00 AM). All procedures were carried out in accordance with NIH Animal Care Guidelines and the animal use protocol approved by University of Delaware Institutional Animal Care and Use Committee.

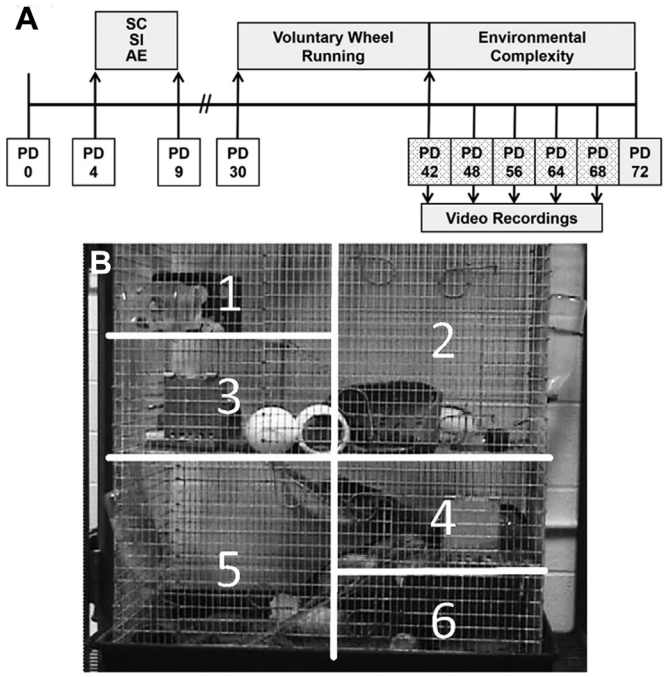

Fig. 1. A) Experimental Timeline. Rats were exposed to 5.25 g/kg/day alcohol in a binge-like manner on PD 4–9 and weaned on PD 23. AE: Alcohol-exposed; SI: Sham-intubated; SC: Suckle control (undisturbed). The rats were then housed 3/cage with voluntary access to a running wheel (WR) on PD 30–42 and then housed in environmental complexity (EC) on PD 42–72. Sessions were taped on PD 42, 48, 56, 64, and 68 following the toy change. B) The EC cage was visually split into 6 quadrants on a computer monitor during video analysis to analyze exploratory behavior.

Neonatal alcohol exposure paradigm

On PD 4–9, AE pups were exposed to alcohol in a binge-like manner (5.25 g/kg/day; Fig. 1A). Alcohol was administered in an 11.9% v/v milk solution in 2 doses, 2 h apart via temporary intragastric intubations. On PD 4, 2 supplemental doses of milk formula were administered, 2 h and 4 h following the second alcohol dose to compensate for reduced calorie intake from the dam by AE pups. For the remaining days (PD 5–9), milk formula was administered only once, 2 h following the second alcohol exposure. Sham-intubated (SI) pups received intubations without milk or alcohol solution. SC animals were undisturbed apart from daily weighing (PD 4–9).

Blood alcohol concentrations (BACs)

On PD 4, blood samples were obtained from SI and AE pups for BAC analysis. Blood was collected via tail clip 90 min following the second alcohol exposure. Samples from the AE group were centrifuged (15,000 rpm/15 min), and the plasma was collected and stored at −20 °C. Plasma was analyzed for BAC using an Analox GL5