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Research report

Acute alcohol exposure during neurulation: Behavioral and brain structural consequences in adolescent C57BL/6J mice

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HIGHLIGHTS

- Acute neurulation stage alcohol exposure affected the behaviors of adolescent male and female mice.
- Sex differences were observed on the elevated plus maze, open field, and in social preferences.
- Neurulation stage alcohol exposure affected behavior in the absence of differences in regional brain volumes.
- Neurulation stage alcohol exposure altered the shapes of the cerebellum, hypothalamus, striatum and corpus callosum.

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ABSTRACT

Prenatal alcohol exposure (PAE) can induce physical malformations and behavioral abnormalities that depend in part on thedevelopmental timing of alcohol exposure. The current studies employed a mouse FASD model to characterize the long-term behavioral and brain structural consequences of a binge-like alcohol exposure during neurulation; a first-trimester stage when women are typically unaware that they are pregnant. Time-mated C57BL/6J female mice were administered two alcohol doses (2.8 g/kg, four hours apart) or vehicle starting at gestational day 8.0. Male and female adolescent offspring (postnatal day 28–45) were then examined for motor activity (open field and elevated plus maze), coordination (rotarod), spatial learning and memory (Morris water maze), sensory motor gating (acoustic startle and prepulse inhibition), sociability (three-chambered social test), and nociceptive responses (hot plate). Regional brain volumes and shapes were determined using magnetic resonance imaging. In males, PAE increased activity on the elevated plus maze and reduced social novelty preference, while in females PAE increased exploratory behavior in the open field and transiently impaired rotarod performance. In both males and females, PAE modestly impaired Morris water maze performance and decreased the latency to respond on the hot plate. There were no brain volume differences; however, significant shape differences were found in the cerebellum, hypothalamus, striatum, and corpus callosum. These results demonstrate that alcohol exposure during neurulation can have functional consequences into adolescence, even in the absence of significant brain regional volumetric changes. However, PAE-induced regional shape changes provide evidence for persistent brain alterations and suggest alternative clinical diagnostic markers.

1. Introduction

It is well known that alcohol exposure during pregnancy can harm the developing fetus. Yet, many pregnant women continue to drink alcohol and alcohol exposure during pregnancy remains the single most preventable cause of birth defects. The constellation of physical and behavioral abnormalities that are caused by prenatal alcohol exposure (PAE) are classified under the term fetal alcohol spectrum disorders (FASDs), which ranges in severity from fetal alcohol syndrome (FAS) to alcohol-related neurodevelopmental disorder (ARND) or neurobehavioral disorder associated with prenatal alcohol exposure (ND-PAE). While distinctive facial features are critical to the diagnosis of FAS, the vast majority of individuals with PAE arenot facially dysmorphic but can nonetheless suffer from persistent and pervasive behavioral impairments, including

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attention, memory, and executive function deficits [33] as well as hyperactivity and motor incoordination [26,22,16]. Additionally,prenatal alcohol-exposed individuals are more susceptible to developing externalizing behavioral and mood disorders related to anxiety, depression, and substance abuse [2,34,40]. Unfortunately, the behavioral outcomes following PAE are extremely variable, frustrating attempts at consistent and comprehensive diagnosis.

The wide variety of physical and behavioral manifestations of FASDs is likely due to the interaction between factors such as genetics, maternal nutrition, and the amount, pattern and timing of alcohol exposure [4,46]. Since many of these variables are difficult to delineate in human studies, animal models are invaluable to understanding specific contributions to the overall FASD phenotype. The C57BL/6J (C57) mouse has proven especially useful for understanding the importance of the developmental timing of early acute binge-like alcohol exposure and studies in this strain have demonstrated that gastrulation and neurulation are two early developmental periods when the embryo is critically sensitive to alcohol [56]. In human development, these periods occur in the middle of the third week of gestation and end of the third week to an early fourth week of gestation, respectively, times before most women realize that they are pregnant. Thus, even if a woman abstains from drinking alcohol once she realizes she is pregnant, inadvertent alcohol exposures during gastrulation and neurulation are likely.

During gastrulation, acute binge-like alcohol exposure can cause the loss of midline facial and brain structures, defects that fall within the holoprosencephaly spectrum [18,17] and are recognized as the classic FAS face [57]. In contrast, alcohol exposure during neurulation induces subtle defects of the fetal face and brain, most notably a smaller cerebellum and right hippocampus, and a relatively expanded hypothalamic/diencephalon area, septal region, pituitary and cerebral ventricles, as well as shape alterations in the cortex, hippocampus, and striatum [45]. The variety of effects of early gestational PAE on the fetal brain suggests that there may also be complementary behavioral changes. Unfortunately, the severity of brain defects following gastrulation PAE precludes a thorough examination because many of these mice do not survive after birth.

A few studies have examined the consequences of acute neurulation-stage alcohol exposure on a limited number of postnatal behavioral outcomes. Some studies have shown evidence for delayed sensorimotor development [13,50], altered exploratory behavior [13] impaired spatial learning or memory during adolescence or adulthood [59,23,38,58], suggesting altered hippocampal function, but others have not [48]. The research described herein was designed to further understand the consequences of neurulation alcohol exposure by examining a comprehensive battery of behavioral tests in adolescent male and female C57 mice that were exposed to acute alcohol injections on GD8, the beginning of neurulation. Based on the structural changes observed in the fetal brain, particularly the expansion of the septum and reduction of the cerebellum and hippocampus, it was hypothesized that GD8 alcohol exposure would affect measures of motor coordination, exploratory behavior, learning, and social behavior. In addition, to determine if the structural changes in the fetal brain would persist into adolescence, brain regional volumes and shapes were analyzed by magnetic resonance imaging.

2. Materials and methods

2.1. Mice

All experiments were conducted following the guidelines of the National Institutes of Health using methods approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill. Female C57BL/6J mice (n = 41 treated dams) were obtained from The Jackson Laboratory (Bar Harbor, ME), weighed approximately 20 g upon arrival, and were housed in groups of five or fewer in standard polycarbonate cages with cob bedding and cotton nesting material. Rodent chow (Isopro RMH 3000; Purina, St. Louis, MO) and tap water were freely available through the cage lid. Timed matings with male C57BL/6J mice began three hours into the light cycle and lasted one to two hours; gestational day 0, 0 h (GD0) was defined as the beginning of the breeding period in which a copulation plug was detected. At GD8, separate groups of pregnant dams were administered two intraperitoneal (i.p.) injections, 4 h apart, of 23.7% (v/v) ethyl alcohol (Pharmaco-Aaper, Brookfield, CT) in a lactated Ringer's solution at a dose of 2.8 g/kg, or the equivalent volume of the vehicle alone. Peak blood alcohol levels following this procedure have been shown to be approximately 380 mg/dl, 30 min after the second alcohol injection [45]. Alcohol- and control- treated litters were housed with their dams, culled to a maximum of 8 pups/litter at postnatal day 3 (PD3), and left undisturbed until weaning at PD28 when they were housed in same sex groups with their litter mates. To control for litter effects, one to two male and female mice were randomly selected from each litter for subsequent behavioral experiments conducted between PD28–45 or for ex vivo imaging conducted at PD45.

2.2. Procedures

Behavioral experiments were conducted every weekday in the UNC Behavioral Phenotyping Core during the light portion of the 12:12 h light:dark schedule by experimenters who were blinded to the treatment conditions. The behavioral testing battery was performed in 14 male (8 litters) and 14 female (8 litters) vehicle-exposed mice and 12 male (8 litters)and 12 female (8 litters) prenatal alcohol-exposed mice. All mice were tested in the following testing order: rotarod trials 1–3; elevated plus maze; open-field; rotarod trials 4 and 5; acoustic startle/pre-pulse inhibition; Morris water maze acquisition; Morris water maze reversal; hot plate. Social behaviors were available from separate mice (Veh males n = 11; PAE males n = 12; Veh females n = 11; PAE females n = 11) tested on PD 28.

2.2.1. Rotarod

To measure motor coordination and balance, mice were placed on a rotating barrel of a rotarod apparatus (Ugo-Basile, Stoelting Co., Wood Dale, II) which progressively accelerated from 3 rpm to 30 rpm during the maximum of a 5-min test. An observer recorded the latency to fall off or rotate around the top of the barrel during three repeated trials on the first day of testing and two repeated trials on the second day of testing. Each trial was separated by about 45 s.

2.2.2. Elevated plus maze

To measure exploration of an environment containing typically preferred and avoided spaces, mice were placed in the center of a metal maze elevated 50 cm above the floor that contained two open arms (30 cm length) and two closed arms (20 cm high walls). During the 5-min test, an observer recorded the number of entries and time spent in each of the arms. These data were used to calculate the percent of open arm time [(open arm time/total arm time) × 100], the total arm entries, and the percent of open arm entries [(open arm entries/total arm entries) × 100].

2.2.3. Open field

To measure spontaneous motor activity and exploration of a novel environment, the mice were placed in the corner of an