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Research report

Effects of sex and housing on social, spatial, and motor behavior in adult rats exposed to moderate levels of alcohol during prenatal development

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HIGHLIGHTS

- Prenatal alcohol exposure negatively affects social, motor, and spatial behavior.
- Robust behavioral effects of ethanol exposure were only observed in males.
- Housing with a non-exposed control rat did not benefit ethanol-exposed males.
- Housing with an ethanol-exposed partner negatively affected non-exposed males.

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ABSTRACT

Persistent deficits in social behavior, motor behavior, and behavioral flexibility are among the major negative consequences associated with exposure to ethanol during prenatal development. Prior work from our laboratory has linked moderate prenatal alcohol exposure (PAE) in the rat to deficits in these behavioral domains, which depend upon the ventrolateral frontal cortex (Hamilton et al., 2014) [20]. Manipulations of the social environment cause modifications of dendritic morphology and experience-dependent immediate early gene expression in ventrolateral frontal cortex (Hamilton et al., 2010) [19], and may yield positive behavioral outcomes following PAE. In the present study we evaluated the effects of housing PAE rats with non-exposed control rats on adult behavior. Rats of both sexes were either paired with a partner from the same prenatal treatment condition (ethanol or saccharin) or from the opposite condition (mixed housing condition). At four months of age (~3 months after the housing manipulation commenced), social behavior, tongue protrusion, and behavioral flexibility in the Morris water task were measured as in (Hamilton et al., 2014) [20]. The behavioral effects of moderate PAE were primarily limited to males and were not ameliorated by housing with a non-ethanol exposed partner. Unexpectedly, social behavior, motor behavior, and spatial flexibility were adversely affected in control rats housed with a PAE rat (i.e., in mixed housing), indicating that housing with a PAE rat has broad behavioral consequences beyond the social domain. These observations provide further evidence that moderate PAE negatively affects social behavior, and underscore the importance of considering potential negative effects of housing with PAE animals on the behavior of critical comparison groups.

1. Introduction

Fetal Alcohol Spectrum Disorders (FASD) include Fetal Alcohol Syndrome (FAS), partial FAS (pFAS), and other disorders for which morphological or behavioral consequences are observed in the context of confirmed prenatal alcohol exposure (PAE) [9,46,57].

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The negative consequences of FASD include, but are not limited to, deficits in social behavior, learning, memory, and cognition [1,23,34,42,43,53,57,58,62,80]. FASD is a world-wide public health problem [59]. Estimates of FASD prevalence rates in the United States occur between 2%–5% [45,48], with a large majority of FASD cases falling within the less severe range of the spectrum [47]. While high levels of PAE may lead to greater morphological, neurological, behavioral, and cognitive deficits, the consequences of moderate PAE lead to more subtle, yet persistent deficits in humans and non-human animals [11,40,64,65,73].

Deficits in social behavior, motor behavior, and spatial learning and memory have been repeatedly observed in children with FASD [23,34,43,53,54,57,80] and in non-human animal models of PAE [4,12,16,17,26,31,51,52,70,72,75,77], though the detection of deficits with more moderate blood ethanol concentrations (BECs) (e.g., BECs of ~60–80 mg/dl) [20,66] or low exposure (e.g., BECs < ~40 mg/dl) [13] may require more challenging tasks compared to heavy exposure (e.g., ~BECs > 200 mg/dl). Prior work from our laboratory reported increased agonistic wrestling (in contrast to play behavior) in male, but not female, rats following moderate PAE [19]. In the same study, the influence of housing with novel partners on PAE-related social behavior was examined by pair-housing PAE or saccharin control rats with novel, untreated rats obtained from a vendor that were regularly changed (every 48 h for 40 days). This manipulation increased wrestling in male and female PAE offspring, suggesting increased aggression toward novel partners. However, the interpretation of these results is complicated by the fact that moderate PAE has more commonly yielded null effects on social behavior in female rats under standard housing conditions [19], and the behavioral phenotype of rats provided by commercial vendors may differ from those of animals bred and reared on site [8]. In a recent study [20] we replicated the effect of moderate PAE on male social behavior and observed impaired motor behaviors (tongue protrusion [78,79]), and decreased behavioral flexibility as measured by increases in perseverative errors in the Morris water task. This collection of deficits was of particular interest based on commonalities in the ventrolateral frontocortical circuitry required for these behaviors and reports of alterations in ventrolateral frontal cortex function following moderate PAE [6,19,22].

Currently, despite considerable need, there are no treatments for the array of behavioral and cognitive deficits associated with FASD. Achieving this goal will critically depend upon the use of animal models to identify mechanisms and evaluate treatment approaches. Alterations in social behavior have been observed in rat models of PAE across a variety of exposure durations, doses, and developmental timing, and are among the most common outcomes observed in FASD [15,18,39]. Thus, social behavior represents an important target for intervention. The social environment and experience in the social domain have been recognized as potential factors that could yield benefits for social behavior as well as other behavioral deficits observed following PAE [30]. For example, Middleton et al. [51] found that PAE on the twelfth day of gestation produced a socially avoidant phenotype that was normalized following housing with a pair of non-exposed control animals. Social manipulations may also, however, promote the expression of atypical social behaviors. Hamilton et al. [19] demonstrated that routinely changing the cage-mate (housing with novel partners every 48 h) from a cohort of non-exposed rats may actually enhance social behavioral alterations following moderate PAE. The goal of the present study was to evaluate the effects of prolonged social housing with a non-exposed cage-mate on long-term social behavioral consequences of moderate PAE, and to determine if the effects of this housing manipulation extend to deficits in motor behavior and behavioral flexibility we have previously reported in male rats following moderate PAE [20]. Because our prior report was limited to male rats, the present study also examined potential sex differences in the effects of moderate PAE and social experience on behavioral outcomes. Offspring of rat dams that voluntarily consumed moderate levels of alcohol throughout pregnancy [24] were pair-housed with a same-sex cage-mate from either the same prenatal treatment condition (PAE or saccharin) or from the opposite condition (mixed housing) until behavioral testing in adulthood. Social behavior, tongue protrusion (TP), and spatial response perseveration errors were quantified as described in Ref. [20].

2. Methods

2.1. Subjects

Subjects were 48 Long-Evans rats (24 male and 24 female) obtained from the University of New Mexico Health Sciences Center Animal Resource Facility (see breeding protocol below). All animals were generated in the same breeding round. After weaning, all animals were housed with a single animal from the same prenatal treatment or the other prenatal treatment condition in standard plastic cages with water and food available ad libitum. All cage-mate pairs were from different litters and matched for age and weight. All animals were at least 4 months of age prior to behavioral testing. Lights were maintained on a reverse 12 h:12 h light:dark cycle with lights off at 0900 h. Approximately 3–4 weeks prior to behavioral testing all rats in this study underwent a single MRI scan under isoflurane anesthesia for ~45 min as part of a separate study. All procedures were approved by the Institutional Animal Care and Use Committee of either the main campus or Health Sciences Center at the University of New Mexico.

2.2. Materials and procedures

2.2.1. Breeding and voluntary ethanol consumption during gestation

All breeding procedures were conducted in the University of New Mexico HSC Animal Resource Facility (ARF). Three to four-month-old Long-Evans rat breeders (Harlan Industries, Indianapolis, IN) were single-housed in plastic cages at 22 °C and kept on a reverse 12-h light/dark schedule (lights on from 2100 to 0900 h) with Purina Breeder Block rat chow and tap water ad libitum. After at least one week of acclimation to the animal facility, all female rats were provided 0.066% saccharin in tap water for four hours each day from 1000 to 1400 h. On Days 1 and 2 the saccharin water contained 0% ethanol. On days 3 and 4 saccharin water contained 2.5% ethanol (v/v). On Day 5 and thereafter saccharin water contained 5% ethanol (v/v). Daily four-hour consumption of ethanol was monitored for at least two weeks and mean daily ethanol consumption was determined for each female breeder. Following two weeks of daily ethanol consumption females that drank at levels more than one standard deviation from the mean of the entire group were removed from the study. The remainder of the females were assigned to either a saccharin control or 5% ethanol drinking group and matched such that the mean pre-pregnancy ethanol consumption by each group was comparable.

Subsequently, females were placed with proven male breeders until pregnant as evidenced by the presence of a vaginal plug. Female rats did not consume ethanol during the breeding procedure. Beginning on Gestational Day 1, rat dams were provided saccharin water containing either 0% or 5% ethanol for four hours a day, beginning precisely at 1000 h (1 h following the onset of the dark cycle). The volume of 0% ethanol saccharin water provided to the controls was matched to the mean volume of 5% ethanol in saccharin water consumed by the ethanol-drinking group, which has remained relatively consistent at about 16 mL per four-hour drink-