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Can low-level ethanol exposure during pregnancy influence maternal care? An investigation using two strains of rat across two generations

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HIGHLIGHTS

- Ethanol and water gavage influence maternal behavior in a strain-specific manner.
- Maternal behavior varies across strains of rat.
- Transgenerational transmission of maternal behavior is strain-specific.
- Prenatal gavage alters transgenerational transmission of maternal behavior.

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ABSTRACT

Gestational alcohol use is well documented as detrimental to both maternal and fetal health, producing an increase in offspring's tendency for alcoholism, as well as in behavioral and neuropsychological disorders. In both rodents and in humans, parental care can influence the development of offspring physiology and behavior. Animal studies that have investigated gestational alcohol use on parental care and/or their interaction mostly employ heavy alcohol use and single strains. This study aimed at investigating the effects of low gestational ethanol dose on parental behavior and its transgenerational transmission, with comparison between two rat strains. Pregnant Sprague Dawley (SD) and Long Evans (LE) progenitor dams (F0) received 1 g/kg ethanol or water through gestational days 17–20 via gavage, or remained untreated in their home cages. At maturity, F1 female offspring were mated with males of the same strain and treatment and were left undisturbed through gestation. Maternal behavior was scored in both generations during the first six postnatal days. Arch-back nursing (ABN) was categorized as: 1, when the dam demonstrated minimal kyphosis; 2, when the dam demonstrated moderate kyphosis; and 3, when the dam displayed maximal kyphosis. Overall, SD showed greater amounts of ABN than LE dams and spent more time in contact with their pups. In the F0 generation, water and ethanol gavage increased ABN1 and contact with pups in SD, behaviors which decreased in treated LE. For ABN2, ethanol-treated SD dams showed more ABN2 than water-treated dams, with no effect of treatment on LE animals. In the F1 generation, prenatal exposure affected retrieval. Transgenerational transmission of LG was observed only in the untreated LE group. Strain-specific differences in maternal behavior were also observed. This study provides evidence that gestational gavage can influence maternal behavior in a strain-specific manner. Our results also suggest that the experimental procedure during gestation and genetic variations between strains may play an important role in the behavioral effects of prenatal manipulations.

1. Introduction

Alcohol is a widely consumed substance with significant health implications, and is particularly detrimental for mother and fetus when consumed during pregnancy. Regardless, epidemiological studies reveal that many women continue drinking during pregnancy despite the consequential increased risk of fetal alcohol syndrome, spectrum disorders, and tendency of alcohol use and abuse for the developing child in the future [1–3]. The Centers for Disease Control and Prevention (CDC) recently stated that 7.6% of pregnant women self-reported alcohol use within the past 30 days, with 1.4% indulging in binge drinking at least once within the period [4]. Evidence of familial transmission of drinking behavior and alcoholism is well established in the literature. Genetic heritability of alcohol use disorders is around 50% in the general population [5]. Children born into families with greater alcohol use also show externalizing behavior problems, which are a risk factor for later

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alcoholism [6]. Interestingly, parental practices moderate these behavioral, cognitive, and psychological effects of prenatal alcohol exposure (PAE) and the familial predisposition to alcoholism in children [7,8]. Parental warmth, consistency in discipline, and parental monitoring decrease the likelihood of behavioral problems in children with biological relatives consuming large quantities of alcohol [8]. This evidence demonstrates the importance of parental care in moderating the later-life consequences of predisposition to alcoholism.

Parental care is affected by exposure to environmental stressors. In humans, it has been suggested that the stress associated with poverty increases parental anxiety and irritability, thus influencing parent–child interactions [9]. In nonhuman primates, environmental adversity increases mother–infant conflicts [10]. In the California mouse, chronic intermittent stress reduces father contact with offspring [11]. The quality and quantity of maternal care are also reduced by chronic stress during gestation in the rat [12]. The prevalence of child abuse and its association with drug use and abuse is well established in the United States. In a 2003 report by the United States Office on Child Abuse and Neglect [13], one-third to two-thirds of child maltreatment cases were related to substance abuse. In fact, children whose parents abuse alcohol and other drugs were three times more likely to be abused and four times more likely to be neglected. Few human studies have looked at the effect of alcohol on human parental behavior, and their results lack consistency. Although Chassin et al. [14] and O'Connor et al. [15] found low-quality parenting in the presence of parental alcoholism, other studies have reported the opposite [16] or no effect [8] of alcohol on parental practices. Thus, there is a need for an improved understanding of the effect of alcohol use on parental behavior.

Animal models may provide much-needed insight. To date, animal models of fetal alcohol spectrum disorders have been widely used; however, few research groups have investigated gestational ethanol exposure effects on maternal behavior. Vorhees [17] used cross-fostering to study the influence of postnatal care on gestational ethanol exposure effects. They found no effect of prenatal ethanol on postnatal care in Long Evans (LE) rats following a liquid diet procedure where pregnant dams were exposed to ethanol throughout gestation. Marino et al. [18] looked at the effect of high doses (intragastric 4.5 g/kg) of ethanol during the entire gestation period on maternal care in LE rats. No effect on maternal behavior was found as a result of the prenatal exposure, although combined pre- and post-natal exposure was shown to increase pups' ultrasonic vocalizations. The occurrence and latency of pup retrieval and nursing posture following low and moderate doses (1 and 2 g/kg, respectively) of ethanol exposure during late gestation were also investigated in Wistar-derived rats [19]. No effects were observed for these two behaviors. However, the maternal care observation period for this study was very short. Thus, more thorough investigation is needed to attest for the lack of effects of gestational ethanol exposure on the maternal behavior of this species. A third group investigated the combined effects of prenatal ethanol and nicotine on maternal behavior [20] and found slight, but significant, decreases in maternal care after this combined treatment. However the effects of nicotine or ethanol alone were not dissected. The present experiment aimed at investigating the effect of a low dose of ethanol exposure during late pregnancy in two strains of rats, the LE and the Sprague Dawley (SD), by performing a more comprehensive analysis of a wider array of behaviors indicative of maternal care.

Clarifying the influence of gestational alcohol exposure on maternal behavior is important, as quality of parental care has been shown to influence offspring development. In primates and rodents, maternal deprivation studies show behavioral and neurophysiological evidence that a lack of appropriate maternal care results in increased anxiety and fearfulness [21–23], increased aggressive behavior [24], and impaired cognitive function in offspring [23,25]. Even slight variations in maternal behavior may have an important impact on the psychological and physiological development of the young. Natural variations in maternal care in the rat are associated with differences in gene expression, in neurotransmitter and hormone release, and in behavior of the offspring [26–31]. The effects of maternal behavior on infant development persist to maturity and influence the offspring's phenotype. More specifically, lower levels of maternal licking/grooming (LG) result in early onset of puberty [26,27], increase stress reactivity [32], influence reproductive strategy [28,30,32,33], and increase alcohol self-administration [34]. Furthermore, maternal behavior has been shown to be transmitted across generations through epigenetic modification, such that female rats that have received low level of maternal care will also provide low levels of maternal care to their offspring [35,36]. Thus, maternal care in the rodent has a large impact on the offspring outcome. Positive parenting practices can actually remedy some of the detrimental effects of prenatal alcohol exposure (PAE), with improvements to both intelligence and coping skills in childhood [15,37]. Additionally, variations in parenting style influence alcohol consumption and abuse in human adolescents and adults [38,39]. Therefore, if alcohol consumption during pregnancy is capable of influencing maternal behavior, it will be important to determine whether variations in maternal care in the rodent mediate the effects of prenatal ethanol exposure on offspring phenotype.

Although rat strains respond differently to similar experimental manipulations, few studies test multiple strains of rat to verify their results. Most researchers will spend their entire research career using the same strain, fearing that changing the animal model can change the effects they have previously found. Genetic variations between strains of rats may influence response to certain tests [40]. Additionally, rat strains have been shown to differ both in voluntary ethanol consumption [41] and in the effects of gestational stress on LG behavior [42]. For translational research purposes, it is vital to verify the validity of results, at least across different strains of the same species. Here we compare the effect of gestational ethanol and water, administered via gavage, on maternal behavior of both SD and LE rats.

An important aim of this study was to model gestational low-level alcohol consumption in humans and its consequences on fetal outcome. Exploring this aim using a rodent model required a paradigm that exposed pregnant dams to a small quantity of ethanol. Hence, we selected a PAE paradigm that has been proven to be effective, over the past two decades, to produce neurophysiological alterations that are similar to most paradigms that used longer durations of exposure to higher doses [42–44,65]. Additionally, late gestation in the rat is a sensitive period during which perturbations may affect later maternal behavior [43]. During late gestation, the hypothalamic oxytocin system undergoes notable changes in preparation for parturition and nursing which are principally regulated by γ-aminobutyric acid and endogenous opioid systems, important targets for ethanol [44,45]. Existing literature suggests that PAE may influence systems important for maternal care, such as oxytocin [20]. Thus, late gestation in the rodent may represent a critical window for the effects of ethanol exposure on future maternal behavior.

Research has shown that exposure to low–moderate doses of ethanol during late pregnancy in SD rats results in an increase in ethanol consumption in offspring [46–48]. Considering that variations in maternal care may influence the offspring's behavior, this experiment also aimed to investigate the effects of gestational ethanol exposure on maternal behavior in two strains of rat. Based on the research findings of Chassin and O'Connor [1,14,15], we predicted that gestational ethanol exposure would decrease levels of maternal behavior. Furthermore, since maternal care has been shown to be transmitted across generations [35], we also investigated the effect of prenatal ethanol exposure on maternal behavior in the adult female offspring, predicting that gestational exposure effects on maternal behavior would be passed on to future generations of mothers.

2. General methods

2.1. Subjects

Thirty-four female Long-Evans (LE) rats were obtained from the Charles River Laboratories (Wilmington, MA, USA) and were allowed