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Third trimester-equivalent ethanol exposure increases anxiety-like behavior and glutamatergic transmission in the basolateral amygdala

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ABSTRACT

Ethanol consumption during pregnancy produces a wide range of morphological and behavioral alterations known as fetal alcohol spectrum disorder (FASD). Among the behavioral deficits associated with FASD is an increased probability of developing anxiety disorders. Studies with animal models of FASD have demonstrated that ethanol exposure during the equivalent to the 1st and 2nd trimesters of human pregnancy increases anxiety-like behavior. Here, we examined the impact on this type of behavior of exposure to high doses of ethanol in vapor inhalation chambers during the rat equivalent to the human 3rd trimester of pregnancy (i.e., neonatal period in these animals). We evaluated anxiety-like behavior with the elevated plus maze. Using whole-cell patch-clamp electrophysiological techniques in brain slices, we also characterized glutamatergic and GABAergic synaptic transmission in the basolateral amygdala, a brain region that has been implicated to play a role in emotional behavior. We found that ethanol-exposed adolescent offspring preferred the closed arms over the open arms in the elevated plus maze and displayed lower head dipping activity than controls. Electrophysiological measurements showed an increase in the frequency of spontaneous and miniature excitatory postsynaptic currents in pyramidal neurons from the ethanol group. These findings suggest that high-dose ethanol exposure during the equivalent to the last trimester of human pregnancy can persistently increase excitatory synaptic inputs to principal neurons in the basolateral amygdala, leading to an increase in anxiety-like behaviors.

1. Introduction

Consumption of ethanol during pregnancy can produce fetal alcohol syndrome, characterized by growth retardation, facial dysmorphology, and central nervous system alterations (Riley et al., 2011). However, in many cases, in utero ethanol exposure results in only a subset of these abnormalities. The range of effects that can be caused by prenatal ethanol exposure is denoted as fetal alcohol spectrum disorder (FASD), whose severity depends on several factors, such as the timing, pattern and dose of ethanol consumed, as well as environmental and genetic factors (Jones, 2011). The central nervous system alterations are among the most severe manifestations of FASD, which significantly decrease quality of life and involve a wide range of processes such as learning, memory, attention, fine motor coordination, judgment, social interaction, and emotional behavior (Riley et al., 2011). Regarding the latter, several studies have demonstrated an association between prenatal ethanol exposure and anxiety disorders (O'Connor and Paley, 2009; Hellemans et al., 2010). For instance, O'Leary et al. (2010) found that ethanol exposure during fetal life significantly increases the likelihood of developing anxiety and depression during childhood.

Studies with animal models of FASD have also shown that prenatal ethanol exposure can produce effects on anxiety-like behavior in offspring. Dursun et al. (2006) found that the offspring of rats exposed to high doses of ethanol (6 g/kg via intragastric gavage between gestational days 7 and 20; blood ethanol concentration (BEC) 0.35 g/dl 3 h after gavage) spend less time in the open arms of the elevated plus maze, an indication of increased anxiety-like behavior. Using the open field test, Zhou et al. (2010) showed that high dose prenatal ethanol exposure (6 g/kg of ethanol via intragastric gavage between gestational days 7 and 20; BEC 3 h after last gavage on gestational day 20 = 0.35 g/dl) increases anxiety in adult rat offspring. Developmental exposure of rats or mice to lower doses of ethanol has been shown to produce a decrease in anxiety-like behavior (0.5 and 4 g/kg of ethanol via intragastric gavage from delivery until weaning; BEC not determined; Carneiro et al., 2005), or no significant change (voluntary drinking of saccharin water containing 5% ethanol two weeks pre-pregnancy and throughout gestation; BEC = 0.08 g/dl; Savage et al., 2010; Staples et al., 2013; and drinking in the dark paradigm using 20% v/v ethanol in water throughout gestation; BEC = 0.125–0.175 g/dl; Boehm et al.,

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2008), or an increase (liquid diet containing 6% v/v ethanol throughout gestation; BEC = 0.03 g/dl; Probyn et al., 2012; Cullen et al., 2013) and to have complex effects that are dependent on the sex of the animal and/or its behavioral activation state prior to testing (liquid diet throughout gestation containing approximately 5% v/v ethanol; BEC ≈ 0.13 g/dl; Osborn et al., 1998; Wilcoxon et al., 2005; Gabriel et al., 2006). Collectively, these findings indicate that ethanol exposure during the 1st and 2nd trimesters of pregnancy can have effects on anxiety-like behavior in rodents and that these effects depend on several factors, including the dose and timing of ethanol administration.

Ethen et al. (2009) reported that some pregnant women abstain during the first two trimesters of pregnancy and start drinking during the 3rd trimester, when it may be erroneously assumed that it is safe to drink because key developmental processes are complete. However, neuronal circuits undergo significant differentiation and refinement during this period and this could make them particularly susceptible to the actions of ethanol. To model exposure during this period, ethanol has been administered during the first 1–2 weeks of life in rats, when the brain growth spurt takes place in these animals (Cudd, 2005). An early study with rats exposed to ethanol between postnatal days (P) 4 and 12 using an artificial rearing procedure (BEC = not determined) found evidence consistent with increased anxiety using the open field test (Kaneko et al., 1996). In contrast, more recent studies failed to detect a significant effect of ethanol. Roskam and Koch (2009) found that injecting rats with a high dose of ethanol at P7 (two doses of 2.5 g/kg i.p. delivered 2 h apart; BEC = not determined but this paradigm is expected to produce high levels, near 0.5 g/dl; Ikonomidou et al., 2000) did not significantly alter performance in the elevated plus maze test during adulthood. Diaz et al. (2014a) found that moderate ethanol exposure in vapor chambers between P2 and P12 (BEC = 0.1 g/dl) does not significantly affect anxiety-like behavior during adolescence in rats. Therefore, whether ethanol exposure during the equivalent to the last trimester of human pregnancy affects anxiety-like behaviors remains an open question.

Several brain regions have been implicated in the generation of anxiety-like behaviors (Adhikari, 2014). Among these, the amygdala has been shown to play a prominent role. The basolateral nucleus of the amygdala (BLA) processes information from the ventral hippocampus and medial prefrontal cortex, and relays this information to the central nucleus of the amygdala and bed nucleus of the *stria terminalis* (Adhikari, 2014). These structures project to hypothalamic and brain stem nuclei that mediate physiological changes associated with anxiety (Adhikari, 2014). Several studies have suggested that alterations in the amygdala may contribute to the increase in anxiety-like behavior associated with FASD. Magnetic resonance imaging scans demonstrated amygdala volume reductions in children and adolescents with FASD (Nardelli et al., 2011). Zhou et al. (2010) found that heavy prenatal ethanol exposure enhances excitability of BLA pyramidal neurons by attenuating $GABA_A$ receptor-mediated inhibition. Cullen et al. (2013) showed that moderate prenatal ethanol exposure increases dendritic spines in the apical dendrite of BLA pyramidal neurons. However, the effects of developmental ethanol exposure on the BLA are not fully understood, particularly the impact of exposure during the 3rd trimester equivalent when neuronal circuits in this brain region undergo profound remodeling (Ehrlich et al., 2012, 2013).

In this study, we used vapor inhalation chambers to repeatedly expose rats to high doses of ethanol during the 3rd trimester-equivalent. We evaluated the impact of this exposure paradigm on anxiety-like behavior using the elevated plus maze. We also studied the effect of ethanol on excitatory and inhibitory synaptic transmission in the BLA using patch-clamp electrophysiological techniques in brain slices. These studies focused on adolescent rats (Spear, 2000) because human studies have found an association between prenatal ethanol exposure and behavioral problems during adolescence (Olson et al., 1997).

2. Materials and methods

Unless indicated, all chemicals and drugs were from Sigma-Aldrich (St. Louis, MO).

2.1. Animals

Animal procedures were approved by the UNM-Health Sciences Center Institutional Animal Care and Use Committee. Pregnant Sprague–Dawley rats were obtained from Harlan (Indianapolis, IN) and arrived at gestational days 12–15. Dams were individually housed, received food and water ad libitum, and had a plastic hut in the cage to reduce stress. Lights were on between 6 am and 6 pm.

2.2. Binge-like ethanol vapor chamber exposure

To model binge-like ethanol exposure during the 3rd trimester equivalent, we exposed dams with their pups from P3–5 from 10 am–1 pm daily using vapor inhalation chambers (Morton et al., 2014). We chose this paradigm because exposure to similar levels of ethanol during the same period of development has been previously shown to cause significant neuronal damage (Kane et al., 2011). Ethanol vapor levels were 7.8 ± 0.13 g/dl (n = 37 rounds of exposure), as measured with a breathalyzer (Intoximeters, St. Louis, MO). Controls were exposed to air only. During the 3 days of exposure, animals were handled for weighing purposes and to check for the presence of milk in the stomach. The average number of pups per litter were 10.5 ± 0.5 (n = 12 litters) and 10.13 ± 0.4 (n = 15 litters) for the control and ethanol groups, respectively. Weights were also measured at P6, 10, 15, and 20. After exposure, offspring were allowed to grow until P36–50, when behavioral and electrophysiological experiments were conducted. Blood collection and determination of serum ethanol levels were performed as previously described (Diaz et al., 2014a). With the exception of blood ethanol determinations, only male rats were used for all experiments.

2.3. Maternal care assessment

Maternal care was assessed as previously described (Champagne et al., 2003). The behavior of the dam was recorded with a digital videocamera for 15 min every hour between 10 am and 5 pm. Dam behavior was independently scored by two investigators every 3 min within each 15 min observation period. The dam behaviors that were recorded were: no contact with pups, licking pups, nursing pups in arched-back position, licking pups while they nursed in arched-back position, nursing while lying over the pups in a blanket posture, and passively nursing the pups while lying on the back or side. The percent of the total observation time that each dam spent on each of these behaviors was determined.

2.4. Elevated plus-maze

This behavioral test was performed on P39–42 rats. The apparatus was made of wood painted with a black non-toxic paint. Overhead lighting was set to ~150 lx. The height of the base of the maze was 21″ from the floor. The closed arms were 52.0 × 10.1 × 35.5 cm, and the open arm 52.0 × 10.1 cm. The center square was 10.1 × 10.1 cm. Animal home cages were moved into the testing room at 4 pm, lights were turned off at 5 pm and testing began at 6 pm. The testing room was equipped with a white noise generating system. Each rat was placed on the maze at the juncture of the closed and open arm facing the open arm. We allowed rats to explore the maze for 10 min, rather than the typical 5 min, to increase the chances of detecting differences between treatment groups, as previously described (Komada et al., 2008); during this time the researcher left the room. An overhead camera (ICD-49 B/W digital video camera, Ikegami Electronics, Maywood, NJ) and Ethovision X-T video-tracking software (Noldus, Leesburg, VA) were