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Short communication

Acute exposure to ethanol on gestational day 15 affects social motivation of female offspring

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HIGHLIGHTS

- Prenatal ethanol exposure decreased social investigation in young adolescent females.
- Prenatal ethanol exposure transformed social preference into social avoidance in adult females.
- Males in late adolescence demonstrated significantly more contact behavior and play fighting than older or younger animals.
- Females did not show age-related changes in social interactions.

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ABSTRACT

Alterations in social behavior are a hallmark of many neurodevelopmental disorders in humans. In rodents, social behavior is affected by prenatal insults. The outcomes are dependent on the timing of the insult as well as the sex and age of the animal tested. The limbic system is particularly important for social behavior, and a peak of neurogenesis within this system occurs on gestational day (G)15. Neurons appear particularly vulnerable to ethanol insult around the time they become post-mitotic. We tested the hypothesis that acute exposure to ethanol on G15 would result in significant social behavior deficits. Accordingly, Long Evans pregnant females were injected with ethanol (2.9 g/kg) or an equivalent volume of saline on G15. Offspring were assessed in a modified social interaction test on postnatal day (P) 28, P42, or P75, i.e., during early adolescence, late adolescence, or young adulthood. Prenatal ethanol exposure decreased social investigation in P28 females and transformed social preference into social avoidance in 75-day-old females. Contact behavior, play fighting, and locomotor activity differed as a function of age, but were not significantly affected by ethanol exposure. Males demonstrated significantly more contact behavior and play fighting at P42 than at P28 or P70, whereas there were no age-related changes in females. Adult females showed more locomotor activity than adult males. Overall, prenatal ethanol exposure on G15 enhanced social anxiety in females, with these effects seen in adulthood only.

Alterations in social behavior are among symptoms of many neurodevelopmental disorders, including fetal alcohol spectrum disorder (see [1] for references and review). Preclinical research has shown that acute or chronic prenatal exposure to ethanol alters social behavior of offspring [2–6]. These alterations are dependent on the timing of the exposure as well as the sex and age of the animal tested.

Many neural systems are implicated in the regulation of social behavior, with the limbic system playing a substantial role. The limbic system includes the hippocampus, septal nuclei, nucleus accumbens, amygdala, and cortical regions often including orbitofrontal cortex (OFC), occipital and temporal cortices, and the anterior cingulate cortex. Together, the OFC, amygdala, and anterior cingulate cortex have been termed the "social brain", with these structures playing an important role in processing of social information and making decisions regarding social behavior [7].

During development of the central nervous system, neurons pass through critical periods of vulnerability to ethanol, with the time during which neurons undergo their final mitoses being one of such periods (e.g., [8]). In the rat, neurons of the OFC are born between gestational day (G) 13 and G20, with the majority born

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between G15 and G17 [9]. Neurons of the amygdala are born between G12 and G19, with the most neurons produced between G14 and G16 [10]. Many neurons of the ACC are also generated around G15 [11]. Therefore, the present study investigated possible alterations in social behavior following exposure to a high dose of ethanol on G15 during the peak neuronal generation for brain structures implicated in regulation of social behavior.

Pregnant Long Evans rats (Harlan, Indianapolis, IN) were received on G4. G1 was defined as the first day on which a sperm-positive plug was seen. Animals were maintained on a 12/12 h light/dark cycle (lights off at 07:00) in a temperature-controlled (22 °C) facility that was accredited by Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). All procedures were approved by the Committee for Humane Use of Animals (SUNY Upstate Medical University) and the Institutional Animal Care and Use Committee (Syracuse Veteran's Affairs Medical Center).

On G15, pregnant rats were injected with 2.9 g/kg ethanol intraperitoneally (20% (v/v) solution in physiological saline, 0.9% (w/v), pH 7.4) at 9.00 AM (EtOH-exposed). Two hours later, they received a second intraperitoneal injection of 1.45 g/kg ethanol [5,6,8]. Control females received isovolumetric saline injections at the same times (Sal-exposed). Previous work with this model shows that the mean (±standard error) blood ethanol concentration is 287 ± 3.5 mg/dl two hours after the first injection [5].

On postnatal day (P) 3, litters were weighed and culled to 8–10 animals. Pups remained with their biological dam until weaning on P21 and were then housed in same sex groups of three to four littermates. Animals underwent social interaction (SI) testing on P28, P42, or P75 as previously described [5,6]. Briefly, experimental rats (Sal- or EtOH-exposed prenatally) were placed alone in a partitioned testing apparatus for 30 min the day prior to SI testing. On the test day, experimental subjects (Sal- and EtOH-exposed) were marked with Sharpie™, put alone in a standard housing cage for 30 min, and then placed in the testing chamber for five minutes. Test chambers were made of Plexiglas and were 30 cm × 20 cm × 20 cm for adolescents (P28 and P42) and 45 cm × 30 cm × 20 cm for adults (P75). A semi-circular aperture (7 cm × 5 cm for adolescents and 9 cm × 7 cm for adults) in a central clear Plexiglas partition allowed animals to move between compartments. A prenatally non-exposed partner (not marked with Sharpie™), matched for age, sex, and weight, was also placed into the apparatus, and social interactions were recorded for 10 min. The test was performed under dim light conditions.

The frequencies of social investigation, play fighting and contact behavior were assessed from video recordings by an observer without knowledge of exposure condition of any given animal. Social investigation was defined as sniffing of any part of the partner's body. Contact behavior consisted of social grooming and crawling over or under the partner. Play fighting was scored as a sum of following, chasing, playful nape attacks, and pinning. Social motivation was assessed using a coefficient of social preference/avoidance by scoring the number of crossovers (movements between compartments) an experimental subject made toward and away from the test partner. Coefficient (%) = (crossovers to − crossovers from)/(crossovers to + crossovers from) × 100. Social preference was defined by positive values of the coefficient, whereas social avoidance was indicated by negative values. In addition, the total number of crossovers was used as an index of general locomotor activity under social test circumstances.

Data were analyzed using separate for each behavioral measure 3 (age: P28, P42, P75) × 2 (prenatal exposure: Sal, EtOH) × 2 (sex) analyses of variance (ANOVAs). In order to avoid inflating the possibility of type II errors on tests with at least 3 factors [12], Fisher's planned pairwise comparison test was used to explore significant

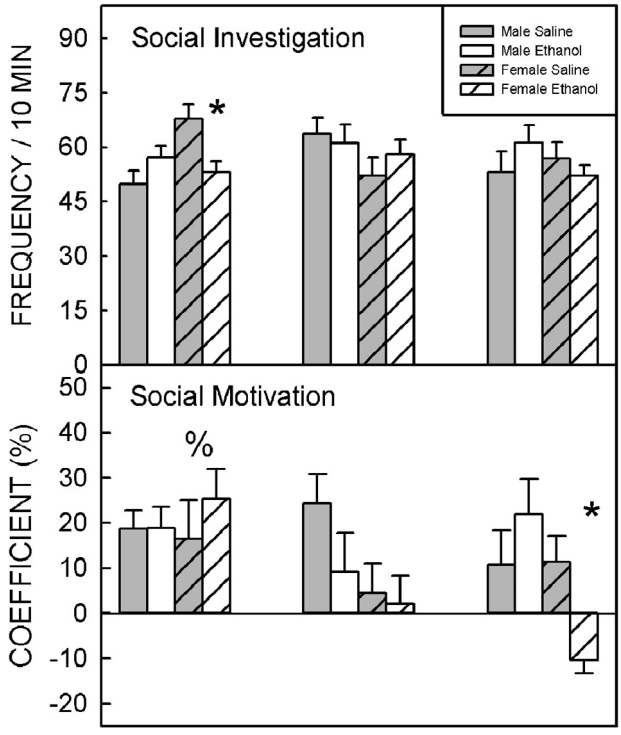

Fig. 1. Social investigation and social motivation showed significant sex × prenatal exposure × age interactions. Social investigation was significantly lower in EtOH-exposed 28-day-old females relative to Sal-exposed counterparts. Within females, social motivation was highest at P28, and by P75 EtOH-exposed females showed social avoidance. Bars show the mean values, *t*-bars are the standard error of the mean. *, EtOH-exposed animals are significantly ($p < 0.05$) different from Sal-exposed age- and sex-matched controls; %, significantly ($p < 0.05$) different to females at other ages.

effects and interactions. Significance was set at $p < 0.05$, and all data are expressed as mean ± standard error ($M \pm SEM$).

1.1. Maternal and litter data

The average daily weight gain of dams did not differ between Sal- and EtOH-injected pregnant females, nor was dam weight on G21 different between these groups (371.6 g ± 6.7 in Sal-exposed dams vs. 355.6 g ± 10 in EtOH-exposed dams). There were no significant differences in the size of the litter or the proportion of males in the litters (Table 1). There was no difference in average pup weight between Sal- or EtOH-exposed groups.

1.2. Social behavior

Social investigation and social motivation showed a significant age × sex × prenatal exposure interaction ($F_{2, 115} = 3.23$, $p = 0.041$ for social investigation; $F_{2, 115} = 3.69$, $p = 0.028$ for social motivation; Fig. 1). Prenatal ethanol exposure significantly decreased social investigation in 28-day-old females relative to their Sal-exposed counterparts, but no effect was seen in 42- or 75-day-old animals. Social motivation was not affected by prenatal ethanol exposure in 28- or 42-day-old females, whereas it substantially decreased the coefficient in 75-day-old females, transforming social preference into social avoidance. The coefficient of social preference was not affected by prenatal exposure to ethanol in male rats at any age.

Contact behavior, play fighting, and locomotor activity were not significantly altered by prenatal ethanol exposure, however significant sex × age interactions were identified for all three measures ($F_{2, 115} = 6.31$, $p = 0.004$ for contact behavior; $F_{2, 115} = 3.79$, $p = 0.026$