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ACUTE PRENATAL EXPOSURE TO ETHANOL AND SOCIAL BEHAVIOR: EFFECT OF AGE, SEX, AND TIMING OF EXPOSURE

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Abstract

During development of the central nervous system, neurons pass through critical periods of vulnerability to environmental factors. Exposure to ethanol during gastrulation or during neuronal generation results in a permanent reduction in the number of neurons in trigeminal-associated cranial nerve nuclei. Normal functioning of the trigeminal system is required for social behavior, the present study examined the effects of acute prenatal exposure to ethanol on social interactions across ontogeny. Pregnant Long-Evans rats were injected with 2.9 g/kg ethanol (i.p., 20% v/v solution; peak blood ethanol concentrations of ~300 mg/dl) or an equivalent volume of saline on gestational day (G) 7 (gastrulation) or G12 (neuronal generation). Subsequently, social investigation, play fighting, contact behavior, social motivation, and overall locomotor activity in the social context were assessed in male and female offspring during early adolescence, late adolescence, or adulthood, on postnatal day (P) 28, P42, or P75, respectively, using a modified social interaction test. Ethanol exposure on G7 resulted in mild changes of social behavior evident in young adolescents only. In contrast, animals exposed to ethanol on G12 demonstrated pronounced behavioral deficits throughout ontogeny, with deficits being most robust in male offspring. Males exposed to ethanol on G12 showed decreases in social investigation, contact behavior, and play fighting, whereas a decrease in social motivation, i.e., transformation of social preference into social avoidance, was evident at P42 and P75 regardless of sex. These findings show that acute exposure to ethanol alters social behavior, and that the timing of the exposure defines the behavioral outcome.

Keywords

adolescence; autism; fetal alcohol syndrome; gastrulation; sex differences

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INTRODUCTION

Alterations in social behavior are associated with developmental disorders such as fetal alcohol spectrum disorder (FASD). Different aspects of human social behavior have been reported to be affected by prenatal exposure to ethanol (see Kelly et al., 2000 for references and review). For instance, children and young adolescents with prenatal alcohol exposure have difficulties considering the consequences of their actions, understanding social cues, and communicating in social contexts (Kelly et al., 2000; Olson et al., 1998; Thomas et al., 1998). Similarly, older adolescents and adults with FASD experience certain difficulties in interactions with peers, as well as deficits in responsiveness to social cues (LaDue et al., 1992; Streissguth et al., 1991). Social behavior of laboratory rodents is sensitive to prenatal ethanol exposure as well. In rats, chronic fetal exposure to ethanol leads to behavioral abnormalities, including alterations in adolescent-characteristic behaviors (Charles Lawrence et al., 2008; Lugo et al., 2003; Meyer & Riley, 1986; Royalty, 1990), as well as in adult-typical forms of social interactions (Kelly & Dillingham, 1994; Lugo et al., 2003).

Rats engage in distinctive forms of interactive behaviors that include social investigation, contact behavior, and play fighting. Each of these forms of social interactions is characterized by a distinct ethological pattern and ontogeny (Meaney & Stewart, 1981; Panksepp et al., 1984; Thor & Holloway, 1984; Vanderschuren et al., 1997; Varlinskaya & Spear, 2008). Social investigation and contact behavior are evident during the entire life span of rats (Vanderschuren et al., 1997), with social investigation being more pronounced in adults than in adolescents (Varlinskaya & Spear, 2008). In contrast, play fighting shows an inverted U-shape developmental pattern, peaking during adolescence and decreasing dramatically thereafter (Panksepp, 1981; Panksepp & Beatty, 1980; Vanderschuren et al., 1997; Varlinskaya et al., 1999).

Under normal circumstances social behavior relies on at least four sensory systems: vision, olfaction, audition, and somatosensation. In the rodent, somatosensation is particularly important for play fighting. Removal of the parietal cortex, the region containing the somatosensory cortex, alters play behavior (Panksepp et al., 1994). Similarly, anesthetizing the nape of the neck alters play behaviors (Charles Lawrence et al., 2008; Siviý and Panksepp, 1987). In conjunction with the increased frequency of play in ethanol-exposed animals, there is a reduction of neuronal activation in somatosensory cortex as determined by c-fos labeling (Charles Lawrence et al., 2008). This is specific to somatosensory cortex, as other brain regions show either an increase or no change in c-fos immunoreactivity (Charles Lawrence et al., 2008; Hamilton et al., 2010).

The central nervous system exhibits discrete periods, or windows, of vulnerability to alcohol. One such window is gastrulation. In rodents, exposure to ethanol during gastrulation, i.e., on gestational day (G) 7 or G8, results in craniofacial malformations (Da Lee et al., 2004; Dunty et al., 2002; Sulik et al., 1981) reminiscent of those seen in children with fetal alcohol syndrome (FAS) (Jones & Smith, 1973; Lemoine et al., 1968), increases the expression of markers for cell death (Dunty et al., 2001; Kilburn et al., 2006), and alters the number of neurons in select (trigeminal-associated) cranial nerve nuclei (Mooney & Miller, 2007). In addition to gastrulation, the developing trigeminal brainstem experiences a second period of vulnerability to ethanol which coincides with the peak of neuronal generation for those neurons (G12/G13).

Given that the trigeminal-somatosensory system plays an important role in social behavior (Charles Lawrence et al., 2008; Panksepp et al., 1994), we tested the hypothesis that acute exposure to ethanol that disrupts the trigeminal-somatosensory system also affects social behavior. Specifically, the present study focused on the distinct vulnerability periods within a rat model by administering ethanol to pregnant females during either gastrulation on G7 or

neural generation on G12. Social behavior of offspring was tested using a modified social interaction test that provided the experimental subject an opportunity to move away from the partner, thereby allowing the assessment of different form of social activity, as well as motivation for social contacts (Varlinskaya et al., 1999, 2001). Male and female rats were tested either as early adolescents on postnatal day (P)28, late adolescents (P42), or young adults on P75 (Spear, 2000; Varlinskaya & Spear, 2008) to determine whether exposure to ethanol during gastrulation or early neural generation results in age- and/or sex-specific alterations in social behavior.

MATERIALS AND METHODS

Subjects

Pregnant Long Evans rats were obtained from Harlan (Indianapolis, IN) on G4. The first day on which a sperm-positive plug was identified was designated G1. Rats were housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited temperature-controlled (22°C) facility and were maintained on a 12/12-hr light/dark cycle (lights off at 0700).

On G7 or G12, females were injected intraperitoneally (i.p.) with 2.9 g/kg ethanol (20% v/v ethanol in physiological saline) at 9.00 AM. Two hours later, animals received a second i.p. injection of 1.45 g/kg ethanol (Mooney & Miller, 2007). Control animals received two i.p. injections of equivalent volumes of saline.

Litters were culled to 10 pups within 24 hr of birth, maintaining a 1:1 sex ratio when possible. Pups were left with their dams in standard plastic maternity cages with pine shavings as bedding material. Animals were weaned on P21 and placed into standard plastic cages with same-sex littermates (5 animals per cage). In all respects, maintenance and treatment of the animals were in accord with guidelines for animal care established by the National Institutes of Health and all procedures were approved by the Committee for the Humane Use of Animals at Upstate Medical University or the Institutional Animal Care and Use Committee at the Syracuse Veteran's Affairs Medical Center.

A total of 40 litters provided 240 male and female offspring to serve as experimental subjects. An additional 28 untreated litters provided 240 animals to serve as partners for the social interaction test. Animals were tested either on P28 (early adolescence), P42 (late adolescence), or P75 (young adulthood). Equal number of males and females ($n = 10$ per group) were placed into each prenatal treatment/age condition to allow analysis of sex effects. To eliminate the possible confounding of litter with prenatal treatment effect, one male and one female subject from each litter was randomly chosen for testing at each age (Holson & Pearce, 1992; Zorrilla, 1997). Weight differences between test subjects and their partners were minimized as much as possible, with this difference not exceeding 10 g at P28 or P42 and 15 g at P75.

Blood Ethanol Concentration

Mean maternal blood ethanol concentration (BEC) was determined using the Analox GM7 (Analox Instruments, Lunenburg MA). Tail blood was collected at various time-points (0.5 – 8 hr) after ethanol injection on G12 from five animals not subsequently used for behavioral experiments.

Social Interaction Test

All testing was conducted under dim light in Plexiglas test apparatuses (30 × 20 × 20 cm for adolescents (P28 and P42) and 45 × 30 × 20 cm for adults (P75); Binghamton Plate Glass, Binghamton, NY). Each test apparatus was divided into two equally sized compartments by a

clear Plexiglas partition that contained an aperture (7×5 cm for adolescents and 9×7 cm for adults) to allow movement of the animals between compartments in a way that only one animal was able to move through the aperture at a time (Varlinskaya et al., 1999; 2001).

One day before testing (P27, P41, or P74), experimental animals were placed alone into a testing apparatus for 30 min to make the testing situation familiar to them, since social interactions are notably higher when animals are tested in a familiar testing chamber than in an unfamiliar chamber (File & Hyde, 1978; File & Seth, 2003). On the test day, each experimental animal was marked on its side with indelible ink, then placed alone in a holding cage for 30 min. This pre-test social deprivation in a novel environment is a standard procedure that has been extensively used to increase baseline levels of social behavior from which inhibitory effects of different experimental manipulations may be more readily detected (File, 1993). The animal was then placed into the testing apparatus. Five min later a non-manipulated partner (i.e., the offspring of a non-treated dam) was also placed into the testing apparatus. Partners were always age-, and sex-matched animals that had not been socially isolated before testing and that were unfamiliar with both the test apparatus and experimental animals with which they were paired for testing (Varlinskaya & Spear, 2002; 2006; 2008). During the 10-min social interaction test session, the behavior of the animals was videotaped for later scoring. After each test, the apparatus was wiped with 3% hydrogen peroxide.

Behavioral Measures

The frequency of a number of social activities (Meaney & Stewart, 1981; Thor & Holloway, 1984; Vanderschuren et al., 1997; Varlinskaya et al., 1999, 2001) of each test subject was analyzed from video recordings. Five behavioral measures were determined: social investigation, contact behavior, play fighting, social motivation, and locomotor activity.

Social investigation was defined as the sniffing of any part of the body of the partner.

Frequency of contact behavior was scored as the sum of crawling over and under the partner and social grooming.

Play fighting was analyzed by scoring the frequencies of the following behavioral acts and postures: pouncing or playful nape attack (the experimental subject lunges at the partner with its forepaws extended outward); following and chasing (the experimental animal rapidly pursues the partner); pinning (the experimental subject stands over the exposed ventral area of the partner, pressing it against the floor). Play fighting differs from serious fighting in the laboratory rat by target of attack: during play fighting snout or oral contact is directed toward the partner's nape, while during serious fighting the object of the attack is the partner's rump (Pellis & Pellis, 1987). In adult animals, serious fighting is characterized by threat postures – a sideways or an upright stance with head movements toward the partner with attempts to bite (offensive sideways or upright posture) and “serious” attacks – a fierce lunging at the partner's rump often associated with biting (Blanchard & Blanchard, 1977). In the present experiments, subjects did not demonstrate threats or serious attacks; therefore, frequency of aggressive behavior (serious fighting) was not scored.

Social motivation was analyzed by scoring the number of crossovers (movements between compartments) demonstrated by the experimental subject toward the partner and the number of crossovers away from the partner (Varlinskaya et al., 1999; 2001), i.e., was expressed as a coefficient of social preference/avoidance [Coefficient (%) = (crossovers to – crossovers from)/(crossovers to + crossovers from)]. Social preference was defined by positive values of the coefficient, whereas social avoidance was associated with negative values.

Locomotor activity was assessed as the total number of crossovers (movements between compartments) demonstrated by each experimental subject. This gives an index of general locomotor activity in this social context (Varlinskaya & Spear, 2002).

Behavioral data were scored from the videotape records by a trained observer who was blind to the prenatal treatment, age, and sex of any animal.

Experimental Design and Data Analysis

The design of the study was a 2 (time of prenatal exposure: G7 or G12) $\times$ 2 (prenatal exposure: saline or ethanol) $\times$ 3 (test age: P28, P42, P75) $\times$ 2 (sex) factorial, with 10 experimental subjects placed into each of the 24 groups defined by this factorial design. Frequency of social investigation, contact behavior, play fighting, coefficient of social preference/avoidance, and total number of crossovers were examined separately for G7 or G12 exposure by using separate 2 (prenatal exposure) $\times$ 3 (test age) $\times$ 2 (sex) between group analyses of variance (ANOVAs), with Fisher Least Significant Difference (LSD) tests used as post hoc comparisons to determine the locus of significant main effects and interactions. Changes in social behavior and social motivation induced by prenatal ethanol exposure were assessed by post-hoc comparisons between ethanol-exposed groups and their age-matched saline-exposed controls.

RESULTS

Blood ethanol concentrations

BECs were determined in a group of five dams that were not used for subsequent testing. Two injections of ethanol were administered as described on G12. Thirty minutes after the first injection of ethanol, mean ($\pm$ standard error of the mean; sem) BEC was 233 ± 7.5 mg/dl. BEC rose to reach the highest value (287 ± 3.5 mg/dl) immediately after the second injection of ethanol, i.e., two hours after the first injection of ethanol. By four hours post-injection, BEC was declining (215 ± 22.1 mg/dl) and eight hours after the first injection of ethanol mean BEC was 1.7 ± 6.2 mg/dl.

Maternal and litter data

Dams were weighed periodically. The mean daily weight gain was recorded for all groups (untreated, saline-, or ethanol-treated on G7 or G12). A one-way ANOVA did not identify any significant differences among the groups treated on G7 ($F_{2,21} = 2.457$; $p = 0.112$) or those treated on G12 ($F_{2,31} = 0.0696$; $p = 0.933$). The mean ($\pm$ sem) daily weight gain for all animals was 5.377 ± 0.182 g.

Exposure to ethanol did not alter the number of pups per litter [mean $\pm$ sem; 11.60 ± 0.28] (Table 1). In most groups, the proportion of male off-spring was slightly higher than female (overall ~55% of pups were male), with no significant differences among the treatment groups. Mean pup weight was determined on P3 and was not altered by prenatal treatment.

Social Investigation

Exposure to ethanol on G7 resulted in mild alterations of social investigation that were only observed in early adolescent animals (Fig. 1). A 2 (prenatal treatment) $\times$ 3 (age) $\times$ 2 (sex) ANOVA revealed a significant prenatal treatment $\times$ age interaction ($F_{2,108} = 4.93$, $p < 0.01$), with social investigation being significantly elevated in ethanol-exposed animals at P28 relative to their saline-exposed counterparts. This effect was not seen in older adolescents and adults.

After exposure to ethanol on G12, the frequency of social investigation differed as a function of age ($F_{2,108} = 4.94$, $p < 0.050$), with older adolescents and adults engaging in more investigation of their partners than younger adolescents. The effects of prenatal ethanol

exposure were also sex-dependent [prenatal treatment $\times$ sex interaction, $F_{1,108} = 6.55$, $p < 0.05$]. Ethanol-exposed males showed significant decreases in social investigation at all ages tested relative to their saline-exposed counterparts. These socially suppressing effects of prenatal ethanol exposure were not seen in females when compared with saline-exposed females of the same age].

Contact Behavior

Ethanol exposure on G7 did not alter contact behavior (Fig. 2). Frequency of contact behavior was also not affected by age or sex. In contrast, after treatment on G12 contact behavior differed as a function of age ($F_{2,108} = 10.75$, $p < 0.001$), with 42-day-old animals being more socially active than older or younger animals. Contact behavior also differed as a function of prenatal exposure and sex [prenatal treatment $\times$ sex interaction, $F_{1,108} = 10.81$, $p < 0.01$]. Specifically, male offspring exposed to ethanol prenatally showed significant decreases in contact behavior relative to saline-exposed males, with these significant decreases seen in early and late adolescents, as well as in adults. Contact behavior of females was not affected by ethanol exposure on G12.

Play Fighting

Animals treated on G7 and tested on P28 showed more play fighting than adult animals [main effect of age, $F_{2,108} = 3.29$, $p < 0.05$; Fig. 3], however this form of social behavior was not affected by prenatal ethanol and did not differ as a function of sex. Interestingly, animals treated on G12 and tested at P42 demonstrated more play fighting than adults [main effect of age, $F_{2,108} = 4.61$, $p < 0.05$]. Ethanol-exposed adolescent and adult males showed significantly less play fighting than their saline-exposed counterparts [prenatal treatment $\times$ sex interaction, $F_{1,108} = 9.46$, $p < 0.01$]. Play fighting of female subjects was not affected by prenatal exposure to ethanol on G12.

Social Motivation

Animals treated on G7 demonstrated social preference at all ages tested, regardless of prenatal treatment, age, or sex (Fig. 4). In contrast, social motivation in both male and female offspring was affected by ethanol exposure on G12. These prenatal effects were age-dependent [prenatal treatment $\times$ age interaction $F_{1,108} = 4.63$, $p < 0.05$]. Saline-exposed controls demonstrated social preference at all ages, as indexed by positive values of the coefficient. No significant changes in social preference induced by prenatal ethanol were seen in animals tested at P28. Post-hoc analyses revealed significant decreases of the coefficient and transformation of social preference into social avoidance in older animals following prenatal exposure to ethanol on G12. This effects was evident in both males and females and was more pronounced in adults than in older adolescents.

Locomotor Activity

Animals treated on G7 showed altered locomotor activity (Fig. 5). There were significant main effects of age [$F_{2,108} = 13.29$, $p < 0.0001$] and sex [$F_{1,108} = 7.92$, $p < 0.01$], as well as a significant age $\times$ prenatal treatment interaction [$F_{2,108} = 4.87$, $p < 0.01$]. In general, adolescents tested at P42 showed significantly more crossovers than their younger and older counterparts, whereas females demonstrated more locomotor activity in the social context than did males. Similarly to social investigation, overall locomotor activity was enhanced by prenatal ethanol exposure in P28 animals, with no effects of prenatal ethanol seen at P42 or P75. In contrast, locomotor activity in the social context did not differ as a function of age or sex and was unaffected by ethanol exposure on G12).

DISCUSSION

Acute exposure to ethanol during prenatal development affected social behavior. Animals exposed to ethanol on G7 showed an increase in social investigation and locomotor activity that was only apparent at early adolescence. The lack of any change in older animals suggests that the ethanol-induced behavioral alterations were compensated later in life. In contrast, exposure to ethanol on G12 caused pronounced and permanent deficits in social behavior throughout ontogeny that were most apparent in male offspring. These animals exhibited a significant reduction of social investigation, contact behavior, and play fighting at all three ages examined. Alterations in social motivation were age-dependent, with transformation of social preference into social avoidance evident in older adolescent and adult males and females. Thus, the social consequences of prenatal ethanol are defined by the timing of ethanol exposure.

Timing of ethanol exposure, along with mean BEC, defines the anatomical outcome. For example, critical periods of vulnerability to ethanol toxicity during which exposure causes neuronal loss have been identified for brainstem (Mooney & Miller 2001; 2007; 2009), somatosensory cortex (compare Miller & Potempa, 1990 with Mooney et al., 1996), and cerebellum (Hamre & West, 1993; Pauli et al., 1995; Pierce et al., 1999). For all these areas, times of relative invulnerability have also been identified. The brainstem exhibits two critical periods (Mooney & Miller, 2007); one is during gastrulation and the second is during the time of peak generation of cranial nerve neurons. Exposure to ethanol at either time results in fewer neurons in trigeminal brainstem nuclei. Exposure to ethanol during gastrulation alters neuronal numbers in the oral portion of the spinal trigeminal nerve and the facial motor nucleus whereas exposure during the peak of neuronal generation results in fewer neurons in the principal sensory nucleus of the trigeminal nerve as well as the trigeminal, facial, and hypoglossal motor nuclei. Intriguingly, deficits in social behavior are only associated with the latter time.

It should be noted that many other systems also underlie social behavior. In the rat, two notable ones are the olfactory system (Sanchez-Andrade and Kendrick, 2009) and the limbic system (Baron-Cohen et al., 1994; Brothers, 1990). The former includes the olfactory epithelium, olfactory bulb, and cortical regions including piriform and entorhinal cortices, the anterior olfactory nucleus, olfactory tubercle and regions of the amygdala. (Sanchez-Andrade and Kendrick, 2009). The olfactory system plays important roles in social recognition (e.g., Carr et al., 1976; Matochick, 1998) and social transmission of food preference, a social communication behavior (Sanchez-Andrade and Kendrick, 2009). The latter includes the orbitofrontal cortex, amygdala, occipital and temporal cortices, and the anterior cingulate cortex (ACC). The combination of these structures has been termed the “social brain” (Baron-Cohen et al., 1994; Brothers, 1990). Many of the above areas are developing during mid-gestation (Altman, 1969; Bayer, 1980; 1983; Bayer and Altman, 1991) and, thus, may be affected by exposure to a teratogen at this time

The lack of any significant differences in mean daily maternal weight gain, in the size or constitution of the litter, or in average pup weight suggests that acute exposure to a high dose of ethanol is not embryo-lethal, nor did it cause significant physiological distress to the dam that interfered with the continuation of the pregnancy. That said, we cannot discount the possibility that injection of ethanol or saline during gestation alters behavior of the dam. Maternal behavior is not significantly altered by chronic exposure to ethanol (Marino et al., 2002). In contrast, pup vocalizations are and this may alter the mother-pup interaction (Kehoe and Shoemaker, 1991; Marino et al., 2002), which, in turn, can affect development of the offspring and their subsequent behavior (for review see Kelly et al., 2009). However, it seems unlikely that the observed social deficits in the offspring were related to alterations in mother-infant interactions, given that behavioral changes in the offspring of the dams acutely exposed to ethanol differed substantially as a function of the timing of exposure.

Behavioral alterations following chronic prenatal/early postnatal alcohol exposure have been reported for a number of social activities, including play fighting (Charles Lawrence et al., 2008; Mayer & Riley, 1986; Royalty, 1990), sexual (Gass et al., 2007), and aggressive behavior (Lugo et al., 2006). In contrast to substantial decreases in social behavior following acute exposure on G12, animals exposed to high doses of ethanol throughout the entire gestational (4.5 g/kg intragastrically) and early postnatal period (3 g/kg ethanol, intragastrically) demonstrate increases in play fighting during adolescence (Charles Lawrence et al., 2008) and enhancement of social investigation during adulthood (Lugo et al., 2003). Enhanced play fighting in adolescent male and female rats has also been reported by Royalty (1990) following shorter and more moderate intragastric maternal ethanol exposure (2 g/kg). In contrast, Meyer and Riley (1986), exposing dams to a liquid diet during gestation, observed decreased play fighting in adolescent males and increased play in their female counterparts. Taken together with the data presented in this paper, the pattern of results clearly demonstrates that alterations in social behavior following prenatal ethanol depend on timing, duration, and level of exposure.

Effects of ethanol exposure on G12 were predominantly sex-dependent, with only male offspring showing decreases in social investigation, contact behavior, and play fighting, whereas ethanol-exposed females did not differ from their saline-exposed counterparts in these measures. Some sex-related differences in the effects of prenatal ethanol exposure on play fighting of adolescent rats have been reported earlier (Meyer & Riley, 1986), with males showing more play fighting than females. The sex differences in baseline levels of play were reversed by prenatal ethanol, that is males became less (feminization) and females became more socially active (masculinization). In contrast, in the present study play fighting of control animals did not differ as a function of sex, with males and females demonstrating comparable levels of play.

Sex differences in social interactions have been reported in some studies for adolescent and adult rats (e.g., Johnston & File, 1991; Pellis & Pellis, 1990; Pellis et al., 1997; Thor & Holloway, 1983). For instance, adolescent males have been shown to engage in more play fighting than females (Meaney 1989; Pellis et al., 1994; Thor & Holloway, 1983), and adult female rats have been reported to spend less time in social interactions relative to their male counterparts (Johnston & File, 1991). In these studies, experimental subjects were socially isolated for at least several days prior to testing, whereas our animals were housed with same-sex littermates and were socially deprived for only 30 min prior to testing. Deprivation from social interactions is stressful, particularly for younger animals (Hall, 1998), and the reported sex-related differences in social behavior may reflect differential sensitivity to such a stressor between males and females (Palanza, 2001). Sex differences in behavioral outcome in the offspring have also been reported following chronic prenatal exposure to ethanol (Hamilton et al., 2010) or chronic prenatal stress (e.g., Weinstock, 2007; Zuena et al., 2008), as well as acute prenatal exposure to other neurotoxicants including chlorpyrifos (Levin et al., 2002) and domoic acid (Levin et al., 2005). The mechanisms underlying these sex-specific effects still remain to be elucidated, but recent evidence from human studies suggest that the level of fetal testosterone can predict sexually differentiated behavior (Auyeung et al., 2009) and is also correlated with alterations in the structure of the brain (Chura et al., 2010).

Gradual changes in social motivation were seen across age in both male and female animals exposed to ethanol on G12. Animals tested at P28 demonstrated social preference and did not differ significantly from their saline-exposed counterparts. Social preference was substantially diminished at P42, whereas adult animals demonstrated pronounced social avoidance of their test partners. Social motivation is extremely sensitive to anxiogenic and/or anxiolytic manipulations in both adolescent and adult animals, with repeated exposure to restraint stress decreasing the coefficient and anxiolytic compounds (including ethanol) increasing social preference (Doremus-Fitzwater et al., 2009; Varlinskaya & Spear, 2002, 2006, 2007).

Therefore, the results of the current study demonstrate age-related increases in anxiety following prenatal ethanol exposure on G12, as indexed by significant decreases of preference/avoidance coefficient (i.e., transformation of social preference into social avoidance) during late adolescence and adulthood. This pattern suggests that increases in social anxiety following acute prenatal ethanol exposure are not the result of the direct effects of ethanol on neural substrates involved in modulation of anxiety-like behavior (Charney, 2003), but rather of persistent social deficits that made the older animals inadequate during social interactions with animals that had regular social experience. Normal social interactions during ontogeny are crucial for establishing social organization within a group or between partners and for the development of the ability to express and understand intraspecific communication signals (Vanderschuren et al., 1997). In the present study, ethanol-exposed animals with social deficits were housed together and were deprived, to some extent, from regular social experience. The lack of this social experience made social interactions with a “normal” partner rather stressful for ethanol-exposed late adolescents and adults, whereas early adolescents still demonstrate social preference, probably due to extremely high social motivation evident at this age (Vanderschuren et al., 1997; Varlinskaya & Spear, 2008).

In summary, in the present study animals exposed to ethanol on G12, but not on G7, demonstrated pronounced social deficits and enhanced anxiety under social circumstances. Behavioral deficits were predominantly seen in male off-spring regardless of age, whereas social anxiety was apparent in older animals regardless of sex. Thus, deficits in social interactions are timing-, sex-, and age-specific. Structural alterations in the “social” brain that underlie these behavioral deficits are remain to be investigated.

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References

- Altman J. Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. *J Comp Neurol* 1969;137:433–457. [PubMed: 5361244]
- Auyeung B, Baron-Cohen S, Ashwin E, Knickmeyer R, Taylor K, Hackett G, Hines M. Fetal testosterone predicts sexually differentiated childhood behavior in girls and in boys. *Psychol Sci* 2009;20:144–148. [PubMed: 19175758]
- Bayer SA. 3H-thymidine-radiographic studies of neurogenesis in the rat olfactory bulb. *Exp Brain Res* 1983;50:329–340. [PubMed: 6641865]
- Bayer, SA.; Altman, J. *Neocortical Development*. Raven Press; New York: 1991.
- Blanchard RJ, Blanchard DC. Aggressive behavior in the rat. *Behav Biol* 1977;21:197–224. [PubMed: 562152]
- Charles Lawrence R, Cale Bonner H, Newsom RJ, Kelly SJ. Effects of alcohol exposure during development on play behavior and c-Fos expression in response to play behavior. *Behav Brain Res* 2008;188:209–218. [PubMed: 18160143]
- Charney DS. Neuroanatomical circuits modulating fear and anxiety behaviors. *Acta Psychiatr Scand Suppl* 2003;417:38–50. [PubMed: 12950435]
- Chura LR, Lombardo MV, Ashwin E, Auyeung B, Chakrabarti B, Bullmore ET, Baron-Cohen S. Organizational effects of fetal testosterone on human corpus callosum size and asymmetry. *Psychoneuroendocrinology* 2010;35:122–132. [PubMed: 19833443]
- Da Lee R, Rhee GS, An SM, Kim SS, Kwack SJ, Seok JH, Chae SY, Park CH, Yoon HJ, Cho DH, Kim HS, Park KL. Differential gene profiles in developing embryo and fetus after in utero exposure to ethanol. *J Toxicol Environ Health Part A* 2004;67:2073–2084. [PubMed: 15513904]

- Doremus-Fitzwater TL, Varlinskaya EI, Spear LP. Social and non-social anxiety in adolescent and adult rats after repeated restraint. *Physiol Behav* 2009;97:484–494. [PubMed: 19345235]
- Dunty WC Jr, Chen SY, Zucker RM, Dehart DB, Sulik KK. Selective vulnerability of embryonic cell populations to ethanol-induced apoptosis: implications for alcohol-related birth defects and neurodevelopmental disorder. *Alcohol Clin Exp Res* 2001;25:1523–1535. [PubMed: 11696674]
- Dunty WC Jr, Zucker RM, Sulik KK. Hindbrain and cranial nerve dysmorphogenesis result from acute maternal ethanol administration. *Dev Neurosci* 2002;24:328–342. [PubMed: 12457071]
- File SE. The social interaction test of anxiety. *Neurosci Protocol* 1993;10:1–7.
- File SE, Hyde JR. Can social interaction be used to measure anxiety? *Brit J Pharmacol* 1978;62:19–24. [PubMed: 563752]
- File SE, Seth P. A review of 25 years of the social interaction test. *Eur J Pharmacol* 2003;463:35–53. [PubMed: 12600701]
- Hall FS. Social deprivation of neonatal, adolescent, and adult rats has distinct neurochemical and behavioral consequences. *Crit Rev Neurobiol* 1998;12:129–162. [PubMed: 9444483]
- Hamilton DA, Akers KG, Rice JP, Johnson TE, Candelaria-Cook FT, Maes LI, Rosenberg M, Valenzuela CF, Savage DD. Prenatal exposure to moderate levels of ethanol alters social behavior in adult rats: relationship to structural plasticity and immediate early gene expression in frontal cortex. *Behav Brain Res* 2010;207:290–304. [PubMed: 19852984]
- Hamre KM, West JR. The effects of the timing of ethanol exposure during the brain growth spurt on the number of cerebellar Purkinje and granule cell nuclear profiles. *Alcohol Clin Exp Res* 1993;17:610–622. [PubMed: 8333592]
- Holson RR, Pearce B. Principles and pitfalls in the analysis of prenatal treatment effects in multiparous species. *Neurotoxicol Teratol* 1992;14:221–228. [PubMed: 1635542]
- Johnston AL, File SE. Sex differences in animal tests of anxiety. *Physiol Behav* 1991;49:245–250. [PubMed: 2062894]
- Jones KL, Smith DW. Recognition of the fetal alcohol syndrome in early infancy. *Lancet* 1973;302:999–1001. [PubMed: 4127281]
- Gass JT, Jenkins WJ, Marino MD, Lugo JN Jr, Kelly SJ. Alcohol exposure during development: analysis of effects on female sexual behavior. *Alcohol Clin Exp Res* 2007;31:2065–2072. [PubMed: 17949467]
- Kehoe P, Shoemaker WJ. Opioid-dependent behavior in infant rats: effects of prenatal exposure to ethanol. *Pharmacol Biochem Behav* 1991;39:389–394. [PubMed: 1946579]
- Kelly SJ, Dillingham RR. Sexually dimorphic effects of perinatal alcohol exposure on social interactions and amygdala DNA and DOPAC concentrations. *Neurotoxicol Teratol* 1994;16:377–384. [PubMed: 7526147]
- Kelly SJ, Day N, Streissguth AP. Effects of prenatal alcohol exposure on social behavior in humans and other species. *Neurotoxicol Teratol* 2002;22:143–149. [PubMed: 10758343]
- Kilburn BA, Chiang PJ, Wang J, Flentke GR, Smith SM, Armant DR. Rapid induction of apoptosis in gastrulating mouse embryos by ethanol and its prevention by HB-EGF. *Alcohol Clin Exp Res* 2006;30:127–134. [PubMed: 16433740]
- LaDue, RA.; Streissguth, AP.; Randels, SP. Clinical considerations pertaining to adolescence and adults with fetal alcohol syndrome. In: Sonderegger, TB., editor. *Perinatal Substance Abuse: Research Findings and Clinical Implications*. Johns Hopkins University Press; Baltimore: 1992. p. 104-133.
- Lemoine PH, Harousseau H, Borteyru JP, Menuet JFC. Les enfants de parents alcooliques: anomalies observées. A propos de 127 cas. *Ouest Medicine* 1968;21:476.
- Lugo JN Jr, Marino MD, Cronise K, Kelly SJ. Effects of alcohol exposure during development on social behavior in rats. *Physiol Behav* 2003;78:185–194. [PubMed: 12576115]
- Lugo JN Jr, Marino MD, Gass JT, Wilson MA, Kelly SJ. Ethanol exposure during development reduces resident aggression and testosterone in rats. *Physiol Behav* 2006;87:330–337. [PubMed: 16336982]
- Marino MD, Cronise K, Lugo JN Jr, Kelly SJ. Ultrasonic vocalizations and maternal-infant interactions in a rat model of fetal alcohol syndrome. *Dev Psychobiol* 2002;41:341–351. [PubMed: 12430158]
- Meaney MJ, Stewart J. Neonatal-androgens influence the social play of prepubescent rats. *Horm Behav* 1981;15:197–213. [PubMed: 7250910]

- Meaney MJ. The sexual differentiation of social play. *Psychiatr Dev* 1989;7:247–261. [PubMed: 2700802]
- Meyer LS, Riley EP. Social play in juvenile rats prenatally exposed to alcohol. *Teratol* 1986;34:1–7.
- Miller MW, Potempa G. Numbers of neurons and glia in mature rat somatosensory cortex: effects of prenatal exposure to ethanol. *J Comp Neurol* 1990;293:92–102. [PubMed: 2312794]
- Mooney SM, Miller MW. Episodic exposure to ethanol during development differentially affects brainstem nuclei in the macaque. *J Neurocytol* 2001;30:973–982. [PubMed: 12626879]
- Mooney SM, Miller MW. Time-specific effects of ethanol exposure on cranial nerve nuclei: gastrulation and neuronogenesis. *Exp Neurol* 2007;205:56–63. [PubMed: 17320867]
- Mooney SM, Miller MW. Vulnerability of macaque cranial nerve neurons to ethanol is time- and site-dependent. *Alcohol* 2009;43:323–331. [PubMed: 19375881]
- Mooney SM, Napper RM, West JR. Long-term effect of postnatal alcohol exposure on the number of cells in the neocortex of the rat: a stereological study. *Alcohol Clin Exp Res* 1996;20:615–623. [PubMed: 8800376]
- Nanson JL. Autism in fetal alcohol syndrome: a report of six cases. *Alcohol Clin Exp Res* 1992;16:558–565. [PubMed: 1626656]
- Newschaffer CJ, Croen LA, Daniels J, Giarelli E, Grether JK, Levy SE, Mandell DS, Miller LA, Pinto-Martin J, Reaven J, Reynolds AM, Rice CE, Schendel D, Windham GC. The epidemiology of autism spectrum disorders. *Ann Rev Public Health* 2007;28:235–258. [PubMed: 17367287]
- Olson HC, Feldman JJ, Streissguth AP, Sampson PD, Bookstein FL. Neuropsychological deficits in adolescents with fetal alcohol syndrome: clinical findings. *Alcohol Clin Exp Res* 1998;22:1998–2012. [PubMed: 9884144]
- Palanza P. Animal models of anxiety and depression: how are females different? *Neurosci Biobehav Rev* 2001;25:219–233. [PubMed: 11378178]
- Panksepp J. The ontogeny of play in rats. *Dev Psychobiol* 1981;14:327–332. [PubMed: 7250521]
- Panksepp J, Beatty WW. Social deprivation and play in rats. *Behav Neural Biol* 1980;30:b197–206.
- Panksepp J, Normansell L, Cox JF, Siviy SM. Effects of neonatal decortication on the social play of juvenile rats. *Physiol Behav* 1994;56:429–43. [PubMed: 7972392]
- Panksepp J, Siviy S, Normansell L. The psychobiology of play: theoretical and methodological perspectives. *Neurosci Biobehav Rev* 1984;8:465–492. [PubMed: 6392950]
- Pauli J, Wilce P, Bedi KS. Acute exposure to alcohol during early postnatal life causes a deficit in the total number of cerebellar Purkinje cells in the rat. *J Comp Neurol* 1995;360:506–512. [PubMed: 8543655]
- Pellis SM, Pellis VC. Play-fighting differs from serious fighting in both target of attack and tactics of fighting in the laboratory rat *Rattus norvegicus*. *Aggr Behav* 1987;13:227–242.
- Pellis SM, Pellis VC. Differential rates of attack, defense, and counterattack during the developmental decrease in play fighting by male and female rats. *Dev Psychobiol* 1990;23:215–231. [PubMed: 2379760]
- Pellis SM, Pellis VC, McKenna MM. Feminine dimension in the play fighting of rats (*Rattus norvegicus*) and its defeminization neonatally by androgens. *J Comp Psychol* 1994;108:68–73. [PubMed: 8174346]
- Pellis SM, Field EF, Smith LK, Pellis VC. Multiple differences in the play fighting of male and female rats. Implications for the causes and functions of play. *Neurosci Biobehav Rev* 1997;21:105–120. [PubMed: 8994213]
- Pierce DR, Williams DK, Light KE. Purkinje cell vulnerability to developmental ethanol exposure in the rat cerebellum. *Alcohol Clin Exp Res* 1999;23:1650–1659. [PubMed: 10549998]
- Rodier PM, Ingram JL, Tisdale B, Nelson S, Romano J. Embryological origin for autism: developmental anomalies of the cranial nerve motor nuclei. *J Comp Neurol* 1996;370:247–261. [PubMed: 8808733]
- Royalty J. Effects of prenatal ethanol exposure on juvenile play-fighting and postpubertal aggression in rats. *Psychol Rep* 1990;66:551–560. [PubMed: 2349347]
- Sanchez-Andrade G, Kendrick KM. The main olfactory system and social learning in mammals. *Behav Brain Res* 2009;200:323–335. [PubMed: 19150375]

- Schneider T, Przewlocki R. Behavioral alterations in rats prenatally exposed to valproic acid: animal model of autism. *Neuropsychopharmacology* 2005;30:80–89. [PubMed: 15238991]
- Schneider T, Roman A, Basta-Kaim A, Kubera M, Budziszewska B, Schneider K, Przewlocki R. Gender-specific behavioral and immunological alterations in an animal model of autism induced by prenatal exposure to valproic acid. *Psychoneuroendocrinology* 2008;33:728–740. [PubMed: 18396377]
- Siviy SM, Panksepp J. Sensory modulation of juvenile play in rats. *Dev Psychobiol* 1987;20:39–55. [PubMed: 3556783]
- Spear LP. The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev* 2000;24:417–463. [PubMed: 10817843]
- Streissguth AP, Giunta CT. Mental health and health needs of infants and preschool children prenatally exposed to alcohol. *Int J Fam Psych* 1988;9:29–47.
- Streissguth AP, Aase JM, Clarren SK, Randels SP, LaDue RA, Smith DF. Fetal alcohol syndrome in adolescents and adults. *JAMA* 1991;265:1961–1967. [PubMed: 2008025]
- Sulik KK, Johnston MC, Webb MA. Fetal alcohol syndrome: embryogenesis in a mouse model. *Science* 1981;214:936–938. [PubMed: 6795717]
- Thomas SE, Kelly SJ, Mattson SN, Riley EP. Comparison of social abilities of children with fetal alcohol syndrome to those of children with similar IQ scores and normal controls. *Alcohol Clin Exp Res* 22:528–533. [PubMed: 9581664]
- Thor DH, Holloway WR Jr. Social play in juvenile rats: a decade of methodological and experimental research. *Neurosci Biobehav Rev* 1984;8:455–464. [PubMed: 6514252]
- Vanderschuren LJ, Niesink RJ, Van Ree JM. The neurobiology of social play behavior in rats. *Neurosci Biobehav Rev* 1997;21:309–326. [PubMed: 9168267]
- Varlinskaya EI, Spear LP. Acute effects of ethanol on social behavior of adolescent and adult rats: role of familiarity of the test situation. *Alcohol Clin Exp Res* 2002;26:1502–1511. [PubMed: 12394283]
- Varlinskaya EI, Spear LP. Differences in the social consequences of ethanol emerge during the course of adolescence in rats: social facilitation, social inhibition, and anxiolysis. *Dev Psychobiol* 2006;48:146–161. [PubMed: 16489593]
- Varlinskaya EI, Spear LP. Chronic tolerance to the social consequences of ethanol in adolescent and adult Sprague-Dawley rats. *Neurotoxicol Teratol* 2007;29:23–30. [PubMed: 17055219]
- Varlinskaya EI, Spear LP. Social interactions in adolescent and adult Sprague-Dawley rats: impact of social deprivation and test context familiarity. *Behav Brain Res* 2008;188:398–405. [PubMed: 18242726]
- Varlinskaya EI, Spear LP, Spear NE. Social behavior and social motivation in adolescent rats: role of housing conditions and partner's activity. *Physiol Behav* 1999;67:475–482. [PubMed: 10549884]
- Varlinskaya EI, Spear LP, Spear NE. Acute effects of ethanol on behavior of adolescent rats: role of social context. *Alcohol Clin Exp Res* 2001;25:377–385. [PubMed: 11290848]
- Zorrilla EP. Multiparous species present problems (and possibilities) to developmentalists. *Dev Psychobiol* 1997;30:141–150. [PubMed: 9068968]

Figure 1.

Effects of exposure to ethanol on G7 or G12 on social investigation of male and female rats tested as early adolescents (P28), late adolescents (P42) or adults (P75). Social investigation was defined as sniffing of any part of the body of the partner. Exposure to ethanol on G7 increased social investigation in young adolescent animals. In contrast, ethanol exposure on G12 decreased social investigation in male off-spring at all ages examined.

An * depicts significant ($p < 0.05$) differences between age-matched saline- and ethanol-exposed animals. Bars show the mean for each group, t-bars depict the standard error of the mean.

Figure 2.

Effects of exposure to ethanol on G7 or G12 on contact behavior of male and female rats tested as early adolescents (P28), late adolescents (P42) or adults (P75). Contact behavior was defined as social grooming and crawling over or under the partner. It was unaffected by ethanol exposure on G7 (top) but was significantly decreased at all ages in males exposed to ethanol on G12 (bottom). Notations as in Figure 1.

Figure 3.

Effects of exposure to ethanol on G7 or G12 on play fighting as early adolescents (P28), late adolescents (P42) or adults (P75).

Play fighting was defined as a sum of pouncing, chasing, and pinning. Play fighting was not altered by exposure to ethanol on G7. Ethanol exposure on G12 significantly ($p < 0.05$) decreased play fighting in males at all ages examined. Notations as in Figure 1.

Figure 4.

Effects of exposure to ethanol on social motivation.

Movements of test animals towards or away from the novel animal were scored. Coefficient (%) = (crossovers to – crossovers from)/(crossovers to + crossovers from). No sex-effect was apparent for treatment on G7 or G12. Animals treated on G7 showed social preference at all ages, defined as positive values for the coefficient (top). Older adolescent and adult animals exposed to ethanol on G12 (bottom) showed social avoidance (indexed by negative values for the coefficient). Notations as in Figure 1.

Figure 5.

Effects of exposure to ethanol on locomotor activity.

Analysis of the total number of crossovers between the two compartments of the apparatus was used as an index of overall locomotor activity in the social context. Activity was higher in young adolescent animals treated with ethanol on G7 than in the age-matched saline-treated control animals (top). Prenatal treatment on G12 had no effect on locomotor activity (bottom). No sex effects were apparent for either treatment. Notations as in Figure 1.

Table 1

Treatment	Maternal wt gain(g/day)	Number of pups	% male pups	Average pup wt on P3(g)
G7 CH	5.71 (0.39)	11.44 (0.60)	49.80 (4.08)	7.82 (0.18)
G7 Ct	5.75 (0.49)	10.67 (0.84)	56.89 (4.80)	8.21 (0.22)
G7 Et	4.59 (0.17)	11.83 (0.31)	58.65 (5.60)	7.80 (0.27)
G12 CH	5.20 (0.49)	11.87 (0.95)	55.66 (4.03)	8.14 (0.14)
G12 Ct	5.40 (0.44)	12.25 (0.60)	58.66 (4.81)	8.12 (0.16)
G12 Et	5.44 (0.44)	11.80 (0.56)	52.78 (4.70)	7.82 (0.17)

CH – untreated, Ct- saline injected, Et – ethanol-injected. G – gestational day. P – postnatal day. All data are mean (standard error of the mean), n = 6 – 10 per group.