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Enhanced sensitivity to socially facilitating and anxiolytic effects of ethanol in adolescent Sprague Dawley rats following acute prenatal ethanol exposure

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Abstract

Emerging evidence suggests that deficits in social functioning and social anxiety are associated with adolescent alcohol use. Our previous research has shown that acute exposure to a high dose of ethanol on gestational day (G) 12 produces social alterations in adolescent Sprague-Dawley rats. The present study assessed whether these social alterations can affect sensitivity to acute ethanol challenge during adolescence. Pregnant females were exposed intraperitoneally (i.p.) to ethanol (2.5 g/kg followed by 1.25 g/kg in 2 hr) or saline on G12, and their male and female offspring were tested on postnatal day (P) 42. Rats were challenged i.p. with one of four ethanol doses (0, 0.5, 0.75, and 1.0 g/kg), and their social behavior was assessed in a modified social interaction test. Social alterations associated with prenatal ethanol exposure and indexed via decreases of social investigation, social preference, and play fighting were evident in males and females challenged with the 0 g/kg ethanol dose. Acute ethanol increased social investigation, social preference, and play fighting in animals prenatally exposed to ethanol. In contrast, rats prenatally exposed to saline, showing no social facilitation, demonstrated significant ethanol-induced (0.75 and 1.0 g/kg) decreases in social behavior. Given that late adolescents demonstrating social alterations induced by prenatal ethanol exposure become sensitive to the socially anxiolytic as well as socially facilitating effects of acute ethanol, it is possible that the attractiveness of ethanol to these adolescents may be based on its ability to alleviate anxiety under social circumstances and facilitate interactions with peers.

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Keywords

acute ethanol challenge; social behavior; social anxiety; social facilitation; anxiolytic effects of ethanol

Introduction

Alcohol is one of the most widely used substances by American adolescents, with as many as 61% of 12th graders reporting lifetime alcohol use and 16% reporting binge drinking (i.e., drinking patterns that bring blood ethanol concentrations to 80 mg/dL) within the past 2 weeks (Johnston et al., 2017). In 2016, about 7.3 million adolescents (12 to 20 years of age) reported drinking in the past month, including 4.5 million who reported binge alcohol use and 1.1 million who reported heavy alcohol use (binge drinking on 5 or more days in the past month). Thus, about three fifths of current underage drinkers (62.5%) were binge alcohol users (SAMSHA, 2017). These high rates of binge drinking are alarming, since adolescents who engage in even episodic heavy drinking are more likely to develop alcohol use disorders later in life (Bonomo et al., 2004; Grant et al., 2001; Hingson et al., 2006) and to experience a number of long-lasting adverse consequences (Bava & Tapert, 2010; Silveri, 2012). In human research, emerging evidence suggests that problematic drinking among adolescents is related to deficits in social functioning (Hover & Gaffney, 1991) and social anxiety (Buckner et al., 2006; Burke and Stephens, 1999; Stewart et al., 2006). For instance, adolescents identified as problem drinkers report poorer social skills and lower self-esteem than non-problem drinkers (Hover & Gaffney, 1991). Therefore, alcohol may become more appealing for these individuals due to its socially facilitating effects (Beck and Treiman, 1996; Beck et al., 2013; Smith et al., 1995; Treiman and Beck, 1996). Indeed, expectancy for social facilitation from drinking is an important predictor of heavy drinking, with adolescents believing that alcohol will make them more confident and relaxed in a social setting (Mackintosh et al., 2006; Smith et al., 1995). Social anxiety in adolescence is viewed as a significant risk factor for the development of alcohol-related problems and alcohol dependence later in life (Carrigan and Randall, 2003), with other anxiety disorders not playing such a role (Buckner et al., 2008; Kessler et al., 1997). Sensitivity to the anxiolytic effects of ethanol may be enhanced in socially anxious adolescents, given that individuals with social anxiety often drink for self-medication purposes (Carrigan and Randall, 2003; Thomas et al., 2003).

Yet, studies that have directly investigated the socially facilitating and socially anxiolytic effects of ethanol in adolescents with social deficits and social anxiety, respectively, are extremely limited (see Carrigan and Randall, 2003 for references and review), especially among underage youth due to certain ethical considerations. Therefore, animal models of social alterations, including social anxiety are of particular importance, since using these models allows investigators to assess whether these social alterations are associated with enhanced sensitivity to socially anxiolytic and socially facilitating effects of ethanol.

Indeed, ethanol-induced social facilitation is not restricted to human adolescents but can be seen in adolescent rats under normal circumstances (Varlinskaya and Spear, 2002). Young

adolescent rats tested at postnatal day (P) 28 or 35 under familiar, non-anxiety provoking circumstances are uniquely sensitive to the socially activating effects of ethanol administered intraperitoneally (i.p.) in a low dose range (0.5 – 0.75 g/kg) on play fighting (Trezza et al., 2009; Varlinskaya and Spear, 2002, 2006; Willey et al., 2009), with this social facilitation normally not evident in older adolescents (P42) or adults (Varlinskaya and Spear, 2015). However, ethanol-induced facilitation of play fighting can be observed in older adolescents (Varlinskaya et al., 2013) and adults (Varlinskaya et al., 2010) that demonstrate alterations in social responsiveness induced by repeated restraint stress. Specifically, stress-induced social anxiety-like behavior, indexed via decreases in social investigation and social preference, is attenuated by acute ethanol (see Varlinskaya and Spear, 2015 for references and review). These findings indicate enhanced sensitivity to the socially activating and socially anxiolytic effects of ethanol in older adolescents with elevated levels of social anxiety-like alterations.

Social alterations in laboratory rodents can be reliably produced by prenatal ethanol exposure, with these alterations evident in adult as well as adolescent animals (see Marquardt and Brigman, 2016 for references and review). Our previous research has also shown that in the rat acute exposure to a high dose of ethanol on gestational day (G) 12 produces social deficits (i.e., decreases in different forms of social behavior, especially the adolescent-typical behavior of play fighting) and elicits social anxiety-like alterations, indexed via significant decreases in social investigation and/or social preference (Diaz et al., 2016; Middleton et al., 2012; Mooney and Varlinskaya, 2011). This animal model of social alterations was used in the present study to test the hypothesis that adolescent rats with social alterations will become unusually sensitive to the socially facilitating and socially anxiolytic effects of ethanol. We have shown previously, that social alterations induced by ethanol exposure on G12 are sex- and age-related. For instance, when testing occurred on P42, ethanol-exposed males showed significant decreases in social investigation only, whereas their female counterparts demonstrated decreased levels of social investigation, social preference, and play fighting (see Diaz et al., 2016). Thus, the present study was designed to assess whether late adolescent rats (P42) that are not sensitive to the socially facilitating and socially anxiolytic effects of acute ethanol under normal circumstances become sensitive to these effects due to social alterations induced by prenatal ethanol exposure on G12. Social testing occurred under familiar, non-anxiety provoking circumstances, given that no anxiolytic effects of acute ethanol challenge are evident under these circumstances in non-manipulated rats (Varlinskaya and Spear, 2002, 2006).

Materials and Methods

Subjects

Experimental subjects were the offspring of Sprague Dawley rats that were produced by time mating in our colony at Binghamton University; colony originated from animals purchased from Taconic Inc. (Bloomington, PA). Animals were housed in a temperature-controlled (22°C) vivarium, and maintained on a 12:12 h light/dark cycle (lights on at 0700 h) with ad libitum access to food (Purina rat chow) and water. For breeding, adult rats were housed in groups of three females and one male in a big plastic cage during a 4-day period. Vaginal smears were collected every day, with the first day of detectable sperm designated as

G1 (see Mooney and Varlinskaya, 2011). On G12, females were injected i.p. with 2.5 g/kg ethanol (20% v/v ethanol in physiological saline). Two hours later, pregnant females received a second i.p. injection of 1.25 g/kg ethanol. This exposure protocol resulted in peak blood ethanol concentrations (BECs) around 400 mg/dl (Diaz et al., 2016). Control females received two i.p. injections of equivalent volumes of saline. Non-injected dams provided males and females that were used as social partners. Day of birth was considered P0. Litters were culled to 8 pups within 48 hr of birth on P2, 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 (4 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, using protocols approved by the Binghamton University Institutional Animal Care and Use Committee.

A total of 16 dams injected with saline (n=8) or ethanol (n=8) on G12 provided 64 male and 64 female experimental subjects (i.e., 32 males and 32 females for each prenatal condition). An additional 64 males and 64 females derived from 16 non-injected dams were used as partners for social testing. Animals were tested on P42.

Experimental Design

The design was a 2 (prenatal exposure: saline, ethanol) x 4 (ethanol challenge dose) x 2 (sex) factorial, with 8 animals placed into each of the 16 experimental conditions. To avoid the possible confounding of litter with acute ethanol effects (Holson and Pearce, 1992; Zorrilla, 1997), animals were assigned semi-randomly to the challenge dose conditions, with the constraint that no more than one subject of a given sex from a given litter was assigned to a particular dose, with order of testing counterbalanced across litters. Since experimental subjects were exposed to ethanol (or saline) prenatally and then challenged with ethanol (or saline) on P42, the term “ethanol-exposed” or “saline-exposed” signified prenatal exposure, whereas “challenged with ethanol or saline” indicated acute injection of ethanol or saline vehicle on P42.

Ethanol Challenge

On P42, ethanol was injected i.p. as a 12.6% (v/v) solution in saline (0.9%, w/v) at doses of 0, 0.5, 0.75, and 1.0 g/kg. Ethanol challenge dose was varied by altering the volume of the 12.6% solution in order to avoid concentration-induced differences in ethanol absorption rate (see Linakis and Cunningham, 1979). Control animals from both prenatal exposure conditions were injected with isotonic saline at a volume equal to that of the highest dose of ethanol administered (i.e., 1% of animal's body weight). All solutions were administered at room temperature. As in our previous work (Varlinskaya & Spear, 2002, 2006, Varlinskaya et al., 2010), the i.p. route of ethanol administration was employed in this study, given that it produces little variability in blood ethanol levels and has been the most commonly used route of administration in neuropharmacological studies of acute ethanol effects.

Procedure

Social testing occurred on P42. All testing was conducted under dim light in Plexiglas test apparatus (30 × 20 × 20 cm) containing clean shavings. Each test apparatus was divided into two equally sized compartments by a clear Plexiglas partition that contained an aperture (7 × 5 cm) to allow movements of the animal between compartments in a way that only one animal was able to move through the aperture at a time (Middleton et al., 2012; Mooney and Varlinskaya, 2011; Varlinskaya and Mooney, 2014; Varlinskaya et al., 1999, 2001).

On test day, experimental subjects were removed from their home cages, weighed, injected with one of the 4 doses of ethanol, marked by a vertical line on the back, and placed individually into the testing apparatus for 30 min. A social partner of the same age and sex was then introduced for a 10-min test period. Partners were always unfamiliar with both the test apparatus and the experimental animal, were not socially deprived prior to the test and were experimentally and drug-naïve. Weight differences between test subjects and their partners were minimized as much as possible, with this weight difference not exceeding 20 g, and test subjects always being heavier than their partners.

During the 10-min test session, the behavior of the animals was video recorded for later scoring. All testing procedures were conducted between 9:00 and 13:00 h. Trunk blood samples were collected immediately after the test for later determination of blood ethanol content (BEC) and corticosterone (CORT) levels.

Behavioral Measures

As in our previous studies, the frequencies of social investigation and play fighting (Diaz et al., 2016; Middleton et al., 2012; Mooney & Varlinskaya, 2011; Vanderschuren et al., 1997; Varlinskaya & Mooney, 2014; Varlinskaya and Spear, 2002, 2006) were scored from the video recordings by a trained investigator without knowledge of the experimental condition of any given animal. The frequencies, rather than time, were scored and analyzed, given that elementary behavioral acts and postures (i.e., ethogram) demonstrated by experimental subjects are discrete and short lasting, especially in adolescent rats. Therefore, the analysis of frequencies provided better characteristics of social behavior under these test circumstances. In the study, we focused on the social measures that have been shown to be sensitive to the socially facilitating and anxiolytic effects of ethanol, namely social investigation, social preference, and play fighting (see Varlinskaya & Spear, 2015 for references and review). Social investigation was defined as the sniffing of any part of the body of the partner. Play fighting was scored as the sum of 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 and Pellis, 1987). In the present study, subjects were tested during adolescence and did not demonstrate threats or serious attacks; therefore frequency of aggressive behavior (serious fighting) was not scored.

Modification of the social interaction test (Varlinskaya et al., 1999), allowing experimental animal to freely move toward or away from a non-manipulated social partner in a 2-compartment testing apparatus, permitted assessment of social motivation via a preference/avoidance coefficient. The number of crossovers demonstrated by the experimental subject towards, as well as away from, the social partner was measured separately, and a coefficient of preference/avoidance was then calculated [coefficient (%) = (crossovers to the partner – crossovers away from the partner)/(total number of crosses both to and away from the partner) x 100]. Social preference was defined by positive values of the coefficient, whereas social avoidance was associated with negative values (Varlinskaya & Spear, 2010). Total number of crossovers (movements between compartments) was used as an index of general locomotor activity under these circumstances.

Blood Ethanol Concentration

For analysis of BEC, trunk blood samples were collected immediately after behavioral testing using heparinized tubes. Blood samples were then rapidly frozen and maintained at –80°C. Samples were assessed for BECs via headspace gas chromatography using a Hewlett Packard (HP) 5890 series II Gas Chromatograph (Wilmington, DE). At the time of assay, blood samples were thawed and 25- μ l aliquots were placed in airtight vials. Vials were placed in a HP 7694E Auto-Sampler, which heated each individual vial for 8 min and then extracted and injected a 1.0 ml sample of the gas headspace into the chromatograph. Ethanol concentrations in each sample were determined using HP Chemstation software, which compares the peak area under the curve in each sample with those of standard curves derived from reference standard solutions.

Corticosterone Determination

Trunk blood samples were collected using heparinized tubes. Samples were then centrifuged at 2°C for 20 minutes at 3000 rpm, and the plasma frozen and maintained in a –80°C freezer until the time of assay. Plasma corticosterone (CORT) levels were analyzed by radioimmunoassay (RIA) using RIA kits obtained from INCBiomedicals, Inc. (Orangeburg, NY).

Data Analyses

For assessments of social alterations associated with ethanol exposure on G12 in males and females, social investigation, social preference/avoidance and play fighting were analyzed in saline-challenged animals using separate 2 (prenatal exposure) x 2 (sex) analyses of variance (ANOVAs). Then the effects of acute ethanol challenge on social investigation, social preference/avoidance, play fighting, total number of crossovers, BECs and CORT levels were examined using separate 2 (prenatal exposure) x 4 (ethanol challenge dose) x 2 (sex) analyses of variance (ANOVAs). Main effects and interactions involving prenatal exposure and ethanol challenge dose were further explored using Fisher's planned least significant difference (LSD) post hoc tests to avoid inflating the possibilities of Type II errors (see Carmer and Swanson, 1973). These planned tests were focused on comparisons between animals exposed to ethanol prenatally with their saline-exposed counterparts under basal conditions (i.e., acute challenge with 0 g/kg ethanol dose). Responsiveness to acute ethanol

challenge was assessed within each prenatal condition. Significance was set at $p < .05$, and all data are expressed as mean $\pm$ standard error ($M \pm SEM$).

Results

Litter data (Table 1)

Number of pups delivered by each female, male/female ratio, and average pup weight on P2 were compared among non-exposed females and females exposed to saline or ethanol on G12 (Table 1). Number of pups per litter, sex ratio, and average pup weight did not differ as a function of prenatal exposure.

Social Investigation (Figure 1)

The ANOVA of social investigation revealed a significant prenatal exposure $\times$ ethanol challenge dose interaction, $F(3, 112) = 16.53$, $p < .0001$. No main effect or interactions involving sex was evident for this or other behavioral measures. Therefore, data presented in Figures 1 – 3 were collapsed across sex. When challenged with saline, animals prenatally exposed to ethanol demonstrated significantly less social investigation than saline-exposed animals. This social alteration associated with prenatal ethanol was reversed by acute ethanol challenge, with doses of 0.5 and 0.75 g/kg significantly increasing social investigation in ethanol-exposed animals relative to their counterparts injected with 0 ethanol dose and restoring social investigation to levels comparable to those of saline-exposed controls challenged with saline vehicle (see Fig. 1). These ethanol-induced increases were evident exclusively in animals prenatally exposed to ethanol, whereas saline-exposed animals showed inhibition of social investigation at 0.75 and 1.0 g/kg ethanol.

Social Preference/Avoidance (Figure 2)

Similarly to social investigation, a significant prenatal exposure $\times$ ethanol challenge dose interaction, $F(3, 112) = 7.80$, $p < .0001$, was evident for the preference/avoidance coefficient. Under acute saline challenge conditions all experimental subjects showed social preference, however, animals exposed to ethanol on G12 demonstrated significantly lower values of the coefficient relative to their prenatally saline-exposed counterparts. This social alteration was reversed by acute ethanol challenge with 0.5 g/kg. The 0.75 g/kg and 1.0 g/kg ethanol challenge doses significantly decreased the coefficient in saline-exposed animals, but produced no effects on ethanol-exposed rats.

Play Fighting (Figure 3)

The ANOVA of play fighting revealed a significant prenatal exposure $\times$ ethanol challenge dose interaction, $F(3, 112) = 11.05$, $p < .0001$. When challenged with saline, rats prenatally exposed to ethanol showed significantly less play fighting than saline-exposed controls. Acute ethanol (0.5 g/kg) significantly enhanced play fighting in the ethanol-exposed group, whereas 0.75 and 1.0 g/kg ethanol did not affect play fighting in these animals. In contrast, saline-exposed controls showed a significant ethanol-induced inhibition of this adolescent-characteristic form of social interactions at all doses tested.

Locomotor Activity

The analysis of general locomotor activity under social circumstances indexed via total number of crossovers between compartments was not affected by acute ethanol challenge in males and females exposed to either ethanol or saline on G12 (overall mean = 23.9, SEM = 0.7).

Blood Ethanol Concentration (Table 2)

BECs increased in a dose-dependent fashion [main effect of ethanol dose, $F(2, 84) = 691.21$, $p < 0.0001$, and were not affected by prenatal condition. A significant main effect of sex [$F(1, 84) = 12.15$, $p < 0.001$] was also observed, with females having overall slightly lower BECs (45.9 ± 3.9 mg/dl) than males (50.8 ± 3.8 mg/dl) when collapsed across ethanol dose.

Corticosterone Levels (Table 3)

The overall ANOVA of CORT levels revealed significant main effects of sex, $F(1, 112) = 57.23$, $p < 0.0001$, and ethanol dose, $F(3, 112) = 3.62$, $p < 0.05$. As expected, females demonstrated higher CORT levels than males. Given this significant main effect of sex, separate 2 (prenatal exposure) $\times$ 4 (ethanol challenge dose) ANOVAs were conducted within each sex. In males, there was a significant main effect of ethanol challenge dose, $F(3, 56) = 3.14$, $p < 0.05$, with CORT levels significantly decreased following the dose of 0.5 g/kg relative to 0 g/kg dose regardless of prenatal condition. CORT levels of females did not differ as a function of either prenatal condition or ethanol challenge dose.

Discussion

Similar to our earlier findings (Middleton et al., 2012; Mooney and Varlinskaya, 2011; Diaz et al., 2016), i.p. injections of pregnant females with high doses of ethanol on G12 had no adverse effects on litter size, sex ratio, and offspring body weight (see Table 1). Furthermore, adolescent Sprague Dawley rats exposed to ethanol on G12 and tested on P42 demonstrated social alterations when challenged with 0 g/kg ethanol. Specifically, ethanol-exposed animals challenged with saline vehicle showed significant decreases in social investigation, social preference and play fighting relative to saline-exposed controls. In contrast to our previous findings (Diaz et al., 2016), no sex differences for any behavioral measure were evident under 0 g/kg ethanol dose. In that earlier study of Diaz et al. (2016), ethanol-exposed males demonstrated decreases in social investigation at P42, whereas no differences in the levels of play fighting and social preference were evident between ethanol-exposed males and their saline-exposed counterparts. In contrast, adolescent females exposed to ethanol on G12 showed substantial decreases in all forms of social behavior relative to saline-exposed controls, demonstrating social alterations similar to those in the present study. One potential reason for the observed differences is that in the earlier study animals were non-manipulated prior to social testing, whereas the present study tested adolescent males and females following an i.p. injection of saline vehicle – a manipulation that might be a mild stressor. Indeed, CORT levels observed following i.p. saline injections (Willey et al., 2012) are similar to those reported following exposure to mild stressors (Adriani & Laviola, 2000; Ryabinin et al., 1999). Given that prenatal ethanol exposure can enhance stress responsiveness in the offspring (Hellems et al., 2010; Weinberg et al.,

2008), the more pronounced social alterations in ethanol-exposed males in the present study may be related to the combination of prenatal ethanol exposure with a mild stress of saline injection.

Most importantly, the consequences of ethanol exposure on G12 were not limited to alterations in social behavior and social preference; ethanol-exposed males and females also demonstrated substantial changes in responsiveness to acute ethanol challenge. Rats prenatally exposed to ethanol displayed notable increases in social investigation, social preference and play fighting after acute ethanol challenge. In contrast, prenatally saline-exposed controls showed only an age-typical inhibition of social behavior following acute ethanol challenge (see Varlinskaya & Spear, 2006). Age-typical inhibition was specific to social behavior, given that overall locomotor activity indexed via total number of crossovers was not suppressed following acute ethanol challenge. These results demonstrate that rats exposed to ethanol on G12 and tested as adolescents on P42 while demonstrating social anxiety and social deficits became uniquely sensitive to the socially anxiolytic (increases in social investigation and social preference) and socially facilitating (increases in play fighting) effects of acute ethanol.

The anxiogenic-like effects of prenatal ethanol exposure on G12, indexed by reduced baseline levels of social investigation and social preference following acute challenge with saline vehicle on P42, were reversed by acute ethanol challenge, with 0.5 and 0.75 g/kg doses of ethanol significantly increasing social investigation and the dose of 0.5 g/kg ethanol enhancing social preference in ethanol-exposed adolescent rats. Evidence for anxiolytic effects of ethanol was seen in ethanol-exposed adolescents only, with animals exposed to saline on G12 demonstrating no increases in social investigation or social preference following acute ethanol challenge. The latter findings are reminiscent of prior work, where anxiolytic properties of ethanol were not pronounced in non-stressed animals (Varlinskaya et al., 2013), although facilitation of social investigation at the 0.5 g/kg ethanol dose was evident in non-stressed P42 adolescents in our earlier study (Varlinskaya and Spear, 2006). In contrast, enhanced sensitivity to ethanol-induced anxiolysis has been reported in stressed P42 adolescents (Varlinskaya et al., 2013). In that study, P42 adolescent animals demonstrated stress-associated social anxiety-like alterations as indexed by significant decreases in social investigation and social preference, and these anxiety-like social alterations were reversed by acute ethanol (Varlinskaya et al., 2013). Social anxiety-like behavioral alterations induced by prenatal exposure on G12 were reversed by acute ethanol as well, suggesting an enhanced sensitivity to the socially anxiolytic effects of ethanol in these animals. This enhancement of sensitivity to ethanol anxiolysis may be related in part to possible alterations in the GABA_A receptor system that has been shown to contribute to a number of ethanol effects (Enoch, 2008; Kumar et al., 2009; Lobo & Harris, 2008), including its anxiolytic properties (Eckardt et al., 1998).

Animals exposed to ethanol on G12 and tested as late adolescents expressed ethanol-induced facilitation of play fighting that is normally evident in early (P28) and mid (P35) adolescents, but not older adolescents and adults, after administration of low doses of ethanol (Varlinskaya and Spear, 2002, 2006). Indeed, control animals exposed to saline on G12 showed no ethanol-associated facilitation of play fighting. In contrast, play fighting in

control animals was suppressed at all test doses of ethanol. Socially facilitating effects of ethanol have been found to be related to ethanol-induced activation of the endogenous mu opioid receptor (MOR) system (Trezza et al., 2009; Varlinskaya & Spear, 2009) as well as N-methyl-D-aspartate (NMDA) receptor antagonism (Morales et al., 2013), suggesting that these systems might be altered by prenatal ethanol exposure.

Ethanol-induced increases in play fighting, social investigation and social preference in P42 rats exposed to ethanol on G12 were not associated with any increases in locomotor activity under social test circumstances when indexed via total number of crossovers. These results suggest that activating effects of acute ethanol challenge were rather specific for social behavior and do not reflect ethanol-induced general activation. Furthermore, prenatal ethanol exposure-related alterations in sensitivity to the social consequences of acute ethanol are unlikely to be attributable to alterations in ethanol pharmacokinetics, given that BECs did not differ as a function of prenatal exposure, with ethanol- and saline-exposed animals demonstrating comparable BECs.

Some experimental evidence points to an enhanced responsiveness of the hypothalamic-pituitary-adrenal (HPA) axis to different stressors in adult animals exposed to ethanol prenatally (Hellemans et al., 2010; Lee et al., 2000; Weinberg et al., 2008; Wiczorek et al., 2015). Therefore, in the present study, plasma CORT levels were measured in order to assess possible relationships between anxiety-like behavioral alterations, sensitivity to acute ethanol challenge and responsiveness of the HPA axis following prenatal ethanol exposure. Although demonstrating social anxiety-like behavioral alterations (i.e., significant decreases of social investigation and social preference under saline challenge conditions), ethanol-exposed males and females did not significantly differ from their saline-exposed counterparts in terms of CORT levels. These results suggest that under certain experimental conditions CORT levels do not necessarily reflect social anxiety-like behavioral alterations. The lack of differences in CORT levels between socially anxious ethanol-exposed and non-socially anxious saline-exposed rats is in agreement with our previous findings, where male rats intermittently exposed to ethanol during adolescence showed social anxiety-like alterations, but did not differ in terms of CORT levels from their non-anxious controls that were exposed to water during adolescence (Varlinskaya et al., 2014).

It was not surprising that CORT levels were substantially higher in females than in males at P42, since female rats respond to different stressors and drugs of abuse with more pronounced activation of the HPA axis than males (Babb et al., 2014; Doremus-Fitzwater et al., 2009; Handa et al., 1994; Hulshof et al., 2012), and these sex differences become apparent after puberty (see Green and McCormick, 2016 for references and review). In general, pubertal maturation is completed between P34-P36 in females and P42-P44 in males when assessed by vaginal opening and sperm presence, respectively (Vetter-O'Hagen & Spear, 2012). Acute ethanol, like many drugs of abuse and stressors, activates the HPA axis of adult animals, resulting in elevated levels of CORT (Ellis, 1966; Ogilvi & Revier, 1997; Patterson-Buckendahl et al., 2005). In contrast to adults, CORT responses to acute ethanol challenge in adolescents are rather poorly investigated, although significant elevation in CORT levels were evident in adolescent males and females tested at P34-P36 following

an i.p. injection of a dose of 1.5 g/kg ethanol, while a dose of 0.5 g/kg was ineffective (see Willey et al., 2012).

In the present study, neither males nor females showed increases in CORT levels in response to acute ethanol challenge at any ethanol dose relative to saline-injected controls. One of the possible explanations is that the effects of ethanol on the HPA axis interfered with the consequences of exposure to an unfamiliar conspecific, therefore masking ethanol-induced increases in CORT. Another explanation is that only one time point following ethanol injection was assessed, with ethanol effects evident later following administration (see Willey et al., 2012). The most intriguing finding, however, was that males showed decreases in CORT levels following the dose of 0.5 g/kg ethanol relative to saline-injected controls, an effect of acute ethanol not evident in females. Surprisingly, this low dose effect was seen in both ethanol- and saline-exposed males. To our knowledge, this is the first demonstration that acute ethanol is able to blunt CORT response, at least under social test circumstances. This sex-specific effect can be related, at least in part, to ethanol-induced changes in testosterone levels. Cicero and Badger (1997) reported that in Sprague Dawley adult rats, low doses of ethanol significantly increased circulating testosterone, with high doses producing opposite effects. In adult male Wistar rats, acutely administered ethanol produced a 4-fold increase in brain concentrations of testosterone (Alomary et al., 2003). In turn, testosterone can inhibit the HPA axis, whereas estrogen can produce the opposite effect (Handa et al., 1994). It seems likely that the dose of 0.5 g/kg ethanol decreased CORT levels through some testosterone increase in P42 males. However, the exact mechanism of this sex-specific ethanol effect on CORT levels remains to be investigated.

In summary, late adolescent rats that demonstrated social alterations following prenatal ethanol exposure on G12 became uniquely sensitive to the socially facilitating and anxiolytic effects of acute ethanol. To the extent that the present findings are applicable to humans, the observed pattern of ethanol sensitivities associated with pre-existing social deficits and social anxiety may promote high levels of alcohol drinking for its socially anxiolytic and socially facilitating effects during late adolescence.

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Highlights

Acute prenatal exposure to ethanol produces social alterations in adolescent rats.

These alterations are reversed by acute ethanol challenge.

Adolescent rats prenatally exposed to ethanol become sensitive to socially facilitating and anxiolytic effects of ethanol.

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Figure 1.

The impact of prenatal exposure to ethanol on acute ethanol-induced alterations in social investigation demonstrated by P42 adolescents during the 10-min social interaction test. Asterisks (*) indicate ethanol dose differences when compared with the corresponding saline control within each prenatal condition ($p < .05$), whereas a significant difference between ethanol-exposed and saline-exposed animals given 0 g/kg dose ethanol is indicated with # ($p < .05$). Data collapsed across sex.

Figure 2.

The impact of prenatal exposure to ethanol on acute ethanol-induced alterations in social preference demonstrated by P42 adolescents during the 10-min social interaction test. Asterisks (*) indicate ethanol dose differences when compared with the corresponding saline control within each prenatal condition ($p < .05$), whereas a significant difference between ethanol-exposed and saline-exposed animals given 0 g/kg dose ethanol is indicated with # ($p < .05$). Data collapsed across sex.

Figure 3.

The impact of prenatal exposure to ethanol on acute ethanol-induced alterations in play fighting demonstrated by P42 adolescents during the 10-min social interaction test. Asterisks (*) indicate ethanol dose differences when compared with the corresponding saline control within each prenatal condition ($p < .05$), whereas a significant difference between ethanol-exposed and saline-exposed animals given 0 g/kg dose ethanol is indicated with # ($p < .05$). Data collapsed across sex.

Table 1

Litter Data.

Prenatal Exposure	Number of pups/litter	Sex ratio (% males/litter)	Average pup weight on P2 (g)
No Exposure	13.1 ± 0.4	45.2 ± 3.3	8.6 ± 0.3
Saline	13.0 ± 0.9	50.5 ± 4.4	8.9 ± 0.4
Ethanol	14.4 ± 0.6	48.1 ± 5.1	8.7 ± 0.2

Data shown as mean ± standard error of the mean.

Table 2

Blood ethanol concentrations (mg/dl) following acute ethanol challenge

Sex	Ethanol Dose (g/kg)	Saline Exposure on G12	Ethanol Exposure on G12
Male	0.5	19.4 ± 2.1	20.4 ± 1.4
	0.75	52.7 ± 2.8	50.0 ± 2.8
	1.0	80.5 ± 3.1	81.6 ± 2.3
Female	0.5	14.2 ± 2.0	13.5 ± 2.2
	0.75	46.4 ± 1.7	45.6 ± 2.2
	1.0	77.9 ± 3.6	78.5 ± 1.5

Data shown as mean ± standard error of the mean.

Table 3

Corticosterone levels (ng/ml) following acute ethanol challenge.

Sex	Ethanol Dose (g/kg)	Saline Exposure on G12	Ethanol Exposed on G12
Male	0	293.0 ± 58.8	340.9 ± 48.7
	0.5	128.9 ± 26.7*	165.5 ± 41.5*
	0.75	237.8 ± 59.7	271.6 ± 81.9
	1.0	342.3 ± 88.4	248.3 ± 54.6
Female	0	621.3 ± 91.9	541.6 ± 92.9
	0.5	421.3 ± 109.6	521.4 ± 82.9
	0.75	407.3 ± 75.7	596.8 ± 103.6
	1.0	679.3 ± 82.3	640.6 ± 112.0

Data shown as mean ± standard error of the mean.

Asterisks (*) indicate significant ethanol dose differences relative to the corresponding saline control ($p < .05$), with data collapsed across prenatal exposure condition.