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Sex differences in sensitivity to the social consequences of acute ethanol and social drinking during adolescence

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Abstract

In human adolescents, sociable males frequently drink to enhance positive emotional states, whereas anxious females often drink to avoid negative affective states. This study used a rat model of adolescence to provide information regarding possible sex differences in contributors to social drinking. The effects of ethanol (0, 0.5, and 0.75 g/kg) on play fighting and social preference were assessed on P30, P32, and P34 using a within-subject design. Then animals were tested in a social drinking paradigm (P37-P40), with this testing revealing high drinkers and low drinkers. Sex differences in sensitivity to ethanol emerged among high and low drinkers. High socially drinking males, but not females, when tested prior to drinking sessions, showed significant increases in play fighting at both doses. In low drinking males, play fighting was increased by 0.5 g/kg ethanol, whereas the higher dose of 0.75 g/kg produced significant decreases in play fighting. High drinking females initially showed low levels of social preference than high drinking males and low drinking females and were extremely sensitive to ethanol-induced enhancement of this social measure. Low social drinkers, both males and females, were more sensitive to the suppressing effects of ethanol on social preference following 0.75 g/kg ethanol. These findings indicate that during adolescence enhanced sensitivity to the facilitating effects of ethanol on play fighting is associated with heavy drinking among males, whereas low social preference together with high sensitivity to ethanol-induced enhancement of social preference is related to high social drinking in females.

Keywords

Adolescence; Rat; Males; Females; Ethanol Intake; Social Consequences

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1. Introduction

Alcohol is one of the most widely used substances by American adolescents, with as many as 72.3% of 12th graders reporting lifetime alcohol use and 25.2% reporting binge drinking within the past 2 weeks (Johnston et al., 2013). A binge pattern of drinking that brings blood ethanol concentrations to 80 mg% and often corresponds to consuming 5 or more drinks for males, or 4 or more drinks for females, is associated with an increased risk for alcohol abuse and dependence among adolescents (see Courtney & Polich, 2009). A critical question regarding adolescent drinking is why do young people drink and sometimes drink excessively?

Young people drink predominantly in social situations, and the impact of social context on adolescent drinking is viewed as particularly important (Read et al., 2003). Analysis of drinking motives (i.e., reasons for drinking) revealed two distinct types of motives for adolescents who engage in heavy and problematic drinking (Ham & Hope, 2003; Kuntsche et al., 2006). Drinking to enhance positive emotional states is associated with high sociability, high impulsivity, and high levels of novelty and sensation seeking, with adolescent males reporting enhancement motives more frequently than adolescent females (Cooper, 1994). Drinking for coping reasons (i.e., drinking to avoid the experience of negative affective states) is associated with high levels of anxiety, especially anxiety in social situations, with adolescent females reporting coping reasons more frequently than their male counterparts (Comeau et al., 2001). These human data suggest that heavy drinkers among adolescent males may be more sensitive to socially facilitating effects of ethanol, whereas enhanced sensitivity to ethanol-associated anxiolysis may be characteristic of socially anxious adolescent females. Yet, it is difficult to disentangle from such studies whether reported drinking-related reductions in social anxiety reflect actual pharmacological properties of alcohol or are more a function of expectations of anxiolytic effects (Book and Randall, 2002; Sutker et al., 1982).

Much of the human research has also involved self-report questionnaires often given in a single session, which limits causal interpretation of the results. Whether enhanced sensitivity to socially facilitating effects of ethanol serves as a major contributor to heavy drinking in adolescent males, with accentuated sensitivity to anxiolytic effects of ethanol contributing to high intake in anxious adolescent females, has yet to be systematically investigated. These variables are difficult to examine in studies with human youth due to ethical considerations that preclude administration of alcohol to adolescents. Similarities found between human adolescents and adolescents of various mammalian species in terms of developmental history, behavioral changes, as well as neural and hormonal alterations provide reasonable justification for the use of animal models to study contributors to alcohol consumption during adolescence (Spear, 2000, 2011; Spear and Varlinskaya, 2010).

Using a simple animal model of adolescence and a modified social interaction test (see Varlinskaya et al., 1999), we have shown that adolescent rats, similar to their human counterparts, are sensitive to socially facilitating effects of ethanol. Relatively low doses of ethanol injected intraperitoneally (i.p.) enhance social behavior in adolescent rats tested in a familiar environment, with play fighting – an adolescent-typical form of social behavior –

being predominantly activated. Ethanol-induced social facilitation declines during adolescence and is normally not evident by late adolescence or in adulthood (Varlinskaya & Spear, 2002, 2006, 2007; Willey et al., 2009). Using a modification of the social interaction test that allows an experimental animal to freely move toward or away from a non-manipulated social partner in a two-compartment testing apparatus, social motivation can be estimated via calculation of a preference/avoidance coefficient during assessment of social behavior (Varlinskaya et al., 1999). Through use of this modified social interaction test, we have found that under familiar, non-stressful and hence non-anxiety-provoking circumstances, adolescent animals demonstrate substantial social preference (Varlinskaya & Spear, 2002, 2008). Further research has shown that social preference is especially sensitive to anxiogenic manipulations and anxiolytic compounds: significant decreases in social preference are evident following exposure to a stressor, with anxiolytic drugs being able to restore this decreased social preference to the levels demonstrated by non-stressed controls (Doremus-Fitzwater et al., 2009; Morales et al., 2013; Varinskaya & Spear, 2008, 2012; Varlinskaya et al., 2010). These findings suggest that diminished social preference in animals tested under familiar, non-anxiety-provoking circumstances can be viewed as an index of anxiety-like alterations in social interactions (Doremus-Fitzwater et al., 2009; Varinskaya & Spear, 2012; Varlinskaya et al., 2010). These anxiety-like behavioral alterations can be reversed or diminished by acute ethanol challenge (Varlinskaya et al., 2010), suggesting that animals with elevated levels of anxiety-like behavior, indexed via low levels of social preference, are sensitive to the socially anxiolytic effects of ethanol.

It has been shown that high levels of ethanol consumption are not restricted to human adolescents but are also evident in adolescents of other mammalian species, with for instance adolescent rats ingesting more ethanol relative to their body weights than do adults (Doremus et al., 2005; Hargreaves et al., 2011; Schramm-Sapyta et al., 2014; Vetter et al., 2007). However the vast majority of animal models of ethanol intake have tested animals alone under circumstances where they are deprived of social stimuli, and many have also chronically housed the animals in social isolation (see Crabbe et al., 2011 for a review). Assessment of drinking under social circumstances, however, seems of considerable importance, given the prominent role of the social environment on ethanol intake, particularly during adolescence (see Anacker & Ryabinin, 2010 for references and review).

Recent work in our laboratory using adolescent rats (Varlinskaya et al., in press) demonstrated that responsiveness to a social peer predicts ethanol intake in a social setting – circumstances under which drinking typically occurs in human adolescents. High levels of social activity, including play fighting, in males and high levels of social anxiety-like behavior (indexed via low social preference) in females were associated with elevated social drinking. These data support the suggestion that males might be exceptionally sensitive to ethanol-induced social facilitation, ingesting ethanol for its socially enhancing properties, whereas females, especially those with enhanced social anxiety-like behavior, may be responsive to the socially anxiolytic effects of ethanol, ingesting it in order to diminish social anxiety-like behavior (Varlinskaya et al., in press). Given that an ethanol solution sweetened with sucrose and saccharin (“supersac”) was used in that study, the relationship between social responsiveness and “supersac” intake was subsequently assessed in a second experiment. In contrast to sweetened ethanol, there was no relationship between levels of

social activity (that included play fighting) and “supersac” intake in either adolescent males or females, whereas levels of social anxiety-like behavior contributed to “supersac” intake in males, but not females. In contrast to high socially anxious females that demonstrated enhanced intake of sweetened ethanol, high socially anxious males demonstrated the lowest intake of “supersac”. These data clearly demonstrate notable differences in sex- and social behavior-dependent intake patterns between sweetened ethanol and the sweetened solution alone (Varlinskaya et al., in press).

Therefore, the present study used a simple rat model of adolescence in combination with a novel model of social drinking to provide new information regarding possible sex differences in contributors to ethanol intake during adolescence, including sensitivity to the social consequences of acute ethanol. Specifically, this study was designed to test whether elevated ethanol intake in adolescent males is related to enhanced sensitivity to the socially facilitating effects of ethanol (indexed via ethanol-induced increases of play fighting), whereas high level of ethanol intake in females is associated with greater baseline levels of anxiety-like behavior (indexed via low levels of social preference) under social circumstances along with enhanced sensitivity to the socially anxiolytic effects of ethanol (indexed via ethanol-associated increases in social preference).

2. Methods

2.1. Subjects

Adolescent Sprague-Dawley male and female rats bred and reared in our colony at Binghamton University were used. A total of eight litters provided 40 male and female offspring to serve as experimental subjects and 40 to serve as partners. Animals were housed in a temperature-controlled (22°C) vivarium, and maintained on a 12:12 hr light:dark cycle (lights on at 0700 hr) with ad libitum access to food (Purina rat chow) and water. Litters were culled to 10 pups (5 males and 5 females) within 24 hr after birth on P0 and reared until weaning with their mothers in standard plastic maternity cages with pine shavings as bedding material. Rats were weaned on P21 and housed with their same-sex littermates. At all times, rats used in the current study were produced, maintained and treated in accordance with the guidelines for animal care established by the National Institutes of Health, using protocols approved by the Binghamton University Institutional Animal Care and Use Committee.

2.2. Experimental Procedure

On P30, experimental subjects were removed from their home cages, weighed, injected with saline (0.9%, w/v) and placed alone into the social interaction-testing chamber for 30 min. Then a same age and sex test partner from another litter was placed in the apparatus, and social interactions were recorded for 10 min. On P32, experimental animals were injected with 0.5 g/kg ethanol (12.6 %, v/v), whereas a dose of 0.75 g/kg ethanol was administered on P34, given that ethanol-induced facilitation of play fighting is the most pronounced between P28 – P35 (Varlinskaya & Spear, 2002, 2006; Varlinskaya et al., 2001). As described previously, animals were placed alone in a testing apparatus immediately after ethanol administration, and 30 min thereafter were exposed to the same social partner as on

P30 for 10 min. Partners were always non-manipulated animals that had not been socially isolated prior to testing and who were unfamiliar with the experimental animal with which they were paired for testing on P30. Partners were selected semi-randomly, with this selection based on body weights of an experimental subject and its partner. Although weight differences between test subjects and their partners were minimized and did not exceeding 10 g, test subjects were always heavier than their partners. Animals were immediately returned to their home cages after each social interaction test. In order to differentiate experimental animals from their social partners during the test, each experimental animal had a mark on its head or body made with a permanent marker.

2.3. Social Interaction Test

Testing was conducted in Plexiglas (Binghamton Plate Glass, Binghamton, NY) test chambers (30 × 20 × 20 cm) that contained clean pine shavings. The test apparatuses were divided into two compartments by a clear Plexiglas partition containing an aperture (7 × 5 cm) to allow movement of animals between compartments (Varlinskaya et al., 1999). Each 10-min social interaction test session was conducted under dim light (15-20 lux) between 1200 and 1600 hr, with a white noise generator used to attenuate extraneous sounds during testing. The behavior of each pair was recorded by a video camera mounted above the apparatus.

2.4. Behavioral Measures

In this study, we focused on play fighting – an adolescent-typical form of social behavior that is extremely sensitive to ethanol-induced social facilitation, social investigation – a more adult-typical form of social behavior (Varlinskaya & Spear, 2002; 2006, 2007), and social preference, a measure previously shown to be sensitive to anxiogenic manipulations and ethanol-associated anxiolysis (Varlinskaya et al., 2010, 2013) and, therefore, used as an index of anxiety-like behavior under social circumstances. The frequencies of play fighting and social investigation were analyzed from video recordings (Vanderschuren et al., 1997; Varlinskaya and Spear, 2002, 2006, 2008) by a trained experimenter without knowledge of the experimental condition of any given animal. Play fighting was scored as the sum of the frequencies of the following behaviors: pouncing or playful nape attack (experimental subject lunges at the partner with its forepaws extended outward); following and chasing (experimental animal rapidly pursues the partner); and pinning (the experimental subject stands over the exposed ventral area of the partner, pressing it against the floor). Social investigation was defined as the sniffing of any part of the body of the partner.

Measuring the number of crossovers (movements between compartments) demonstrated by the experimental subject towards as well as away from the social partner allowed an assessment of a coefficient of social preference/avoidance [coefficient (%) = (crossovers to the partner – crossovers away from the partner)/(total number of crosses both to and away from the partner) × 100]. Positive values of the coefficient reflected social preference, whereas negative values defined social avoidance (Varlinskaya et al., 1999), with high positive values being an index of low social anxiety-like behavior and low positive values reflecting elevated levels of anxiety-like behavior under social circumstances (Doremus-Fitzwater et al., 2009; Varlinskaya et al., 2010; Varlinskaya et al., 2013).

2.5. Social Drinking

Ethanol intake was assessed using a novel social drinking paradigm whereby animals in each housing group of five same-sex littermates were given simultaneous access to ethanol away from the home cage. Each housing group was given access to 2 bottles containing 10% ethanol in “supersac” solution (3% sucrose + 0.125% saccharin, see Ji et al., 2008) in a novel cage for a 30-min drinking session every day from P37 to P40 (four sessions, one session daily), since our preliminary findings demonstrated that adolescent drinking occurred predominantly during the first 15 minutes of a drinking session. Only four drinking sessions were employed, given that long-lasting ethanol exposure during adolescence can alter social activity and anxiety-like behavior, at least in male subjects (Varlinskaya et al., 2014), with these alterations markedly complicating assessment of social contributors to ethanol intake. Experimental animals were not food or water deprived. Each animal in a group had an individual and distinguishable mark on its head or body made by a permanent marker that allowed later estimation of individual intake of each animal in the group from the video recording of each drinking session. Drinking behavior was characterized by the animal standing on its hind limbs and grasping the drinking tube with its forepaws, and by repetitive, regular small movements of the head up and down. This drinking behavior was distinct and readily distinguishable from brief investigation or sniffing of the drinking tube. Determination of total ethanol consumption per session (i.e., g of ethanol ingested by all cage-mates) and time each rat spent drinking, as assessed by video records of the social drinking session, was used to calculate g/kg ethanol intake for each individual animal using the following formula: (individual g/kg intake = g of ethanol consumed by the group/sum of the time spent drinking by all animals $\times$ time for an individual animal/body weight of respective animal). Our preliminary study demonstrated a significant correlation between time spent drinking and volume of ethanol solution consumed in individually tested adolescent male ($r=0.92$, $p=0.0007$) and female ($r=0.90$, $p=0.0003$), therefore confirming that time spent drinking is a reliable measure for assessment of ethanol intake.

2.6. Blood Ethanol Determination

For analysis of blood ethanol content (BEC), experimental subjects were decapitated immediately after the last drinking test and trunk blood samples were collected 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- μl 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.

2.7. Data Analyses

Animals were divided into high and low social drinkers using a median split of the average ethanol intake across the four drinking sessions within each sex ($n=10$ rats/group). Then

individual and sex differences in ethanol intake across 4 drinking sessions were analyzed using a 2 (sex) X 2 (drinking level: low, high) X 4 (drinking session) mixed-factor analysis of variance (ANOVA), with test day treated as a repeated measure. Since BECs were assessed after the last drinking session, ethanol intake on this last day and BECs were analyzed using separate 2 (sex) X 2 (drinking level) ANOVAs. Pre-drinking sensitivity to effects of ethanol on play fighting, social investigation and social preference were analyzed in low and high drinkers using separate 2 (sex) X 2 (drinking level) X 3 (ethanol dose: 0, 0.5, and 0.75 g/kg) ANOVAs, with ethanol dose treated as a repeated measure. In order to avoid inflating the possibility of type II errors on tests with multiple factors (Carmer & Swanson, 1973), Fisher's planned pairwise comparisons were used to explore significant effects and interactions.

3. Results

The analysis of ethanol intake over days revealed significant main effects of intake level, $F(1, 36) = 90.48, p < .0001$, sex, $F(1, 36) = 28.89, p < .0001$, and day, $F(3, 108) = 9.67, p < .0001$ (see Figure 1). Significant differences between low and high drinking animals, both males and females, were evident at each test day, with females ingesting more ethanol than males. All groups significantly increased their intake from day 1 to day 2, whereas ethanol intake gradually decreased from day 2 to day 4 – decreases seemingly most pronounced in low drinking females.

Ethanol intake during the last drinking session on day 4 did not differ as a function of sex in low drinking adolescents, whereas high drinking females demonstrated significantly higher intake under social circumstances than their male counterparts [sex X intake level, $F(1, 36) = 4.16, p < .05$, see Table 1). BECs assessed immediately after this drinking session showed a similar pattern of sex differences [significant sex X intake level interaction, $F(1, 36) = 4.61, p < .05$], with low drinking animals regardless of sex showing extremely low BECs (see Table 1), whereas BECs of high drinking females were significantly higher than in their male counterparts. In both males and females, ethanol intake on day 4 was correlated significantly with BECs ($r=0.81, p < .0001$ for males and $r=0.90, p < .0001$ for females).

The relationship between the effects of acute ethanol challenge on play fighting and levels of ethanol intake (low versus high drinkers) in a social context (see Figure 2) was sex-dependent [drinking level X sex X ethanol dose, $F(2, 72) = 4.30, p < .05$]. Low drinking males and females showed less play fighting under basal (i.e., 0 g/kg ethanol dose) conditions relative to their high drinking counterparts. Most importantly, high socially drinking males showed significant increases in play fighting relative to their own saline baseline levels when challenged with both doses, an effect not evident in their female counterparts. Whereas play fighting was also increased by 0.5 g/kg ethanol among low drinking males, the higher dose of 0.75 g/kg significantly suppressed play fighting in these males. Adolescent females were insensitive to the stimulatory effects of ethanol on play fighting under these test circumstances.

In contrast, the effects of acute ethanol challenge on social investigation did not differ as a function of drinking level or sex (see Figure 3). High and low drinkers regardless of sex

showed significant increases in social investigation following the dose of 0.5 g/kg, whereas the higher dose of 0.75 g/kg ethanol had no effect on this form of social behavior [main effect of ethanol dose, $F(2, 72) = 24.16$, $p < .0001$].

Ethanol-induced changes in social preference (see Figure 4) differed markedly among low and high drinking males and females [drinking level X sex X ethanol dose, $F(2, 72) = 7.84$, $p < .001$]. High drinking females initially showed elevated levels of anxiety-like behavior, as indexed via significantly lower values of the coefficient of social preference in these females relative to their low drinking counterparts or high drinking males. These females demonstrated substantial increases in social preference following both doses of ethanol, an effect not evident in their male counterparts or in low drinkers of either sex. Social preference was significantly decreased in low social drinkers of both sexes by 0.75 g/kg ethanol.

4. Discussion

Male and female adolescent rats differed notably in the relationship between the impact of ethanol on their social behavior and ethanol intake in a social context. Furthermore, baseline levels of adolescent-typical social behavior (play fighting) versus social anxiety-like behavior (indexed via social preference) were differently associated with high social drinking in males and females. High drinking male adolescents when tested prior to drinking sessions as young adolescents demonstrated pronounced ethanol-induced increases in play fighting, whereas high drinking female adolescents demonstrated social anxiety-like behavior indexed via very low social preference and exhibited decreases in social anxiety-like behavior (i.e., increases in social preference) in response to ethanol. In contrast, the more adult-typical behavior of social investigation (both at baseline and in response to ethanol challenge) was not associated with level of social drinking in either adolescent males or females.

In the present study, sex differences in ethanol intake were evident during adolescence, with females ingesting more ethanol than their male counterparts. These findings are in agreement with results reported in adult rodents, with adult females generally ingesting more ethanol on a gram/kilogram basis relative to their male counterparts (Cailhol and Mormede, 2001; Chester et al., 2006; Doremus et al., 2005; Vetter-O'Hagen et al., 2009) -- sex differences that are evident with unlimited 24-h access (Lancaster et al., 1996), limited access to ethanol in 2-bottle choice situations (Chester et al., 2006) and operant self-administration (Blanchard and Glick, 1995). These sex differences may be related to differences in sensitivity to a number of ethanol effects, with females generally being less sensitive than males (Middaugh et al., 1992; Morales et al., 2014; Varlinskaya et al., 2010).

At least two desired effects of alcohol may contribute to heavy and problematic drinking during adolescence: its ability to facilitate interactions with peers (Mackintosh et al., 2006; Smith et al., 1995) and its properties to alleviate anxiety, perhaps especially anxiety seen in the context of social interactions (Carrigan and Randall, 2003). The results of the present study using a simple rat model of adolescence demonstrate that these two properties of alcohol might play different role in adolescent males and females.

We have shown previously that ethanol-induced social facilitation is not restricted to human adolescents but is also evident in adolescent rats (Varlinskaya & Spear, 2002, 2006, 2007; Willey et al., 2009), with increases in social behavior observed following exposure to relatively low doses of ethanol (0.5 – 0.75 g/kg) that produce BECs in the range of approximately 35 to 75 mg/dl (Varlinskaya & Spear, 2002). No sex differences were seen in the socially facilitating effects of ethanol when animals were tested at P28 (Varlinskaya & Spear, 2006) or at P35 (Varlinskaya & Spear, 2002). In contrast to the previous research, the present study revealed striking sex differences in sensitivity to the activating effects of ethanol on play fighting, with only male adolescents showing this effect and the greatest ethanol social stimulation evident in males that subsequently exhibited high levels of social drinking. One of the possible explanations of the observed sex differences in sensitivity to the socially activating effects of ethanol seen here that were not evident in the earlier studies, is that these earlier studies (e.g., Varlinskaya & Spear, 2002, 2006) may not have been powered sufficiently to reveal potential sex differences in adolescent sensitivity to the facilitating effects of ethanol on play fighting, since only five animals of each sex were included in a given experimental condition. Although, it seems unlikely, given that with this number of males or females per group we have managed to reveal sex difference in sensitivity to the social consequences of ethanol in late adolescent animals tested at P42 (Varlinskaya & Spear, 2006). Therefore, the observed insensitivity of adolescent females to the socially facilitating effects of ethanol on play fighting may be related to a within-subject design applied in the present study, with animals tested three times, whereas between-subject designs with a single test session were used previously. This repeated testing might produce different effect in males and females, making males more sensitive and females less sensitive to ethanol-induced facilitation of play fighting. In contrast to play fighting, no sex differences were evident in the facilitating effects of ethanol on social investigation following repeated social testing, with both males and females demonstrating significant increases in social investigation following a challenge with 0.5 g/kg ethanol – stimulatory effects observed in our earlier studies (Varlinskaya & Spear, 2002). Taken together, these results suggest that different neural mechanisms may be involved in the stimulatory effects of ethanol on play fighting versus social investigation. This possibility seems likely, given that social investigation and play fighting have been shown to reflect behaviorally distinctive and differentially regulated forms of interactive social behaviors with separable ontogenetic patterns (Trezza et al., 2010; Vanderschuren et al., 1997; Varlinskaya and Spear, 2008; Varlinskaya et al., 1999).

Our previous research focusing on neural mechanisms of ethanol-induced facilitation of play fighting has revealed that facilitation of this behavior (but not social investigation) is associated, at least in part, with ethanol-induced activation of the endogenous mu opioid receptor (MOR) system (Varlinskaya & Spear, 2009). This earlier finding was not surprising, given that the MOR system is implicated in modulation of play behavior, with selective MOR agonists increasing play fighting in young adolescent males and antagonists suppressing this form of social behavior (Trezza et al., 2010; Vanderschuren et al., 1997). There is a possibility that the endogenous MOR system of adolescent males is more sensitive to ethanol-associated stimulation than that of adolescent females, although no direct comparisons have ever been made. Alternatively, it is possible that in adolescent

females, saline injection per se may have been sufficient to stimulate the stressor-sensitive endogenous MOR system (Coventry et al., 2001; Drolet et al., 2001; Marinelli et al., 2004), with administration of ethanol producing no further effect on play fighting when compared with the baseline values assessed in the same females following saline administration.

While the endogenous MOR system plays a considerable role in facilitation of play fighting by ethanol (Varlinskaya & Spear, 2009), other neural systems are implicated in regulation of play fighting as well. For instance, social behavior during adolescence can be facilitated by indirect cannabinoid agonists (Trezza & Vanderschuren, 2008a, 2008b), whereas CB1 receptor antagonists are able to alter ethanol-induced facilitation of play behavior during early adolescence (Trezza & Vanderschuren, 2009). Furthermore, the endogenous cannabinoid system is implicated in ethanol intake and reinforcement (Vengeliene et al., 2008), suggesting that this system might be activated differentially by ethanol in adolescent males relative to their female counterparts. There is also some experimental evidence that social neuropeptides, vasopressin and oxytocin, playing a substantial role in regulation of social behavior during adulthood (Veenema and Neumann, 2008), are sex-dependently involved in modulation of play fighting (Bredewold et al., 2014; Veenema et al., 2013). Given that these neuropeptide systems are sensitive to ethanol (McGregor and Bowen, 2012), they may contribute to observed sex differences in sensitivity to ethanol-associated facilitation and inhibition of play fighting as well.

Similar to high drinking adolescent males, low drinking males demonstrated ethanol-induced enhancement of play fighting. However, in contrast to the facilitation seen at both doses in high drinking males, this enhancement was evident in low drinking males at only the lowest dose of 0.5 g/kg ethanol, whereas the higher dose of 0.75 g/kg produced significant decreases in play fighting. The observed pattern of sensitivity to the social consequences of ethanol among low drinking males suggest that these animals are exceptionally sensitive to the socially suppressing and presumably adverse effects of ethanol – effects that might curb intake in these low drinking males. Play fighting in adolescent males is under biphasic control of the N-methyl-D-aspartate (NMDA) NMDA system, given that NMDA antagonists facilitate play fighting at low doses and suppress social behavior at higher doses (Morales et al., 2013b; Siviý et al., 1995). These biphasic effects of NMDA antagonists on play fighting are similar to those induced by ethanol (Varlinskaya & Spear, 2002, 2006). This similarity is not surprising, since acute ethanol inhibits the NMDA function (see Moykkynen & Korpi, 2012). Taken together with the results of the present study, it is likely that low drinking males are exceptionally sensitive to the inhibitory effects of acute ethanol on the NMDA system, with even relatively low doses of ethanol (i.e., 0.75 g/kg) producing NMDA inhibition sufficient enough to produce suppression of play fighting. This enhanced sensitivity of low drinking adolescent males to the suppressing effects of ethanol may be one of the factors that curb their intake under social circumstances. These males may drink less in order to avoid aversive states associated with ethanol. In contrast, adolescent males that are insensitive to the inhibitory effects of acute ethanol while being particularly sensitive to ethanol-associated social facilitation may drink more without experiencing adverse consequences of ethanol.

The results of the present study clearly demonstrate that the relationship between sensitivity to the social consequences of acute ethanol challenge and social drinking differs dramatically in females relative to males during adolescence. High drinking adolescent females showed higher levels of anxiety-like behavior under social circumstances (as indexed via low levels of social preference) when tested under baseline conditions than males and than low drinking females. These high drinking females also exhibited marked decreases in social anxiety-like behavior in response to ethanol challenge, as shown via significant ethanol-related increases in social preference. Taken together, these findings suggest that enhanced sensitivity to the socially anxiolytic effects of ethanol may contribute to the high levels of social drinking seen in adolescent females that express high levels of social anxiety-like behavior under basal test circumstances. In contrast, extremely low levels of anxiety-like behavior combined with enhanced sensitivity to the anxiogenic effects of ethanol, indexed via decreases in social preference, may protect adolescent females from heavy drinking. This hypothesis is confirmed, to some extent, by the pronounced decreases in ethanol intake seen over days in low drinking females, but not in their high drinking counterparts. These low drinking females might form an association between the taste of the solution and the adverse, presumably anxiogenic effects of ethanol – an association that could serve to attenuate further drinking.

The enhanced sensitivity to the socially anxiolytic effects of ethanol in high drinking females may be related, at least in part, to alterations in the gamma-aminobutyric acid type A (GABA_A) receptor system. This receptor system has been implicated in modulation of anxiety (Lydiard et al., 2003; Nemeroff, 2003) and 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). GABA_A receptors with different subunit composition are differentially involved in various ethanol effects. For instance, the alpha 1 subunit plays a role in ethanol-associated sedation and motor impairing (Werner et al., 2006), whereas alpha 2/ alpha 3 subunits are implicated in ethanol-induced anxiolysis (Morris et al., 2006). Although still not tested experimentally, there is a possibility that the expression of alpha 2 subunits are the highest in socially anxious adolescent females and the lowest in their non-anxious counterparts, given that the expression of these subunits is enhanced by prior stress in brain regions associated with anxiety (Jacobson-Pick et al., 2012). To the extent that the enhancement of alpha 2 subunit expression plays a substantial role in anxiety-like behavior and ethanol sensitivity, this enhancement might be one of the mechanisms involved in expression of both the enhanced social anxiety-like behavior and greater sensitivity to the socially anxiolytic effects of ethanol that are evident among high drinking adolescent females.

5. Summary

The results of the present study demonstrate that responsiveness to the social consequences of acute ethanol challenge contribute to the levels of ethanol intake under social circumstances in adolescent rats – circumstances that are most common for adolescent drinking in humans (Read et al., 2003). Enhanced sensitivity to the facilitation of play fighting induced by ethanol is associated with high ethanol intake in adolescent males, suggesting that these males ingest ethanol for its socially enhancing properties. High levels

of social anxiety-like behavior together with enhanced sensitivity to ethanol-induced anxiolysis are associated with high levels of social drinking in adolescent females, suggesting that these females ingest ethanol for its socially anxiolytic effects. To the extent that these experimental findings are applicable to humans (see Comeau et al., 2001), drinking under social circumstances in adolescent males with high baseline levels of play fighting associated with enhanced sensitivity to ethanol-induced facilitation of play fighting as well as in adolescent females with high baseline levels of social anxiety associated with elevated sensitivity to ethanol-induced anxiolysis may provide useful sex-dependent animal models of adolescent social drinking. These models might provide valuable background for systematic assessment of sex-specific mechanisms involved in heavy social drinking during adolescence and creation of gender-specific prevention and intervention strategies.

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Highlights

- > Social contributors to adolescent social drinking are sex-dependent.
- > Pronounced ethanol-induced facilitation of play is seen in high drinking males.
- > Pronounced social anxiety-like behavior is evident in high drinking females.
- > High drinking females are sensitive to the socially anxiolytic effects of ethanol.

Figure 1. Ethanol intake under social circumstances across the four drinking sessions in low and high drinking adolescent males and females.

Figure 2.

Changes in play fighting induced by 0.5 and 0.75 g/kg ethanol (within-subject design) in adolescent males (right) and females (left) that subsequently demonstrated low and high ethanol intake under social circumstances.

(*) - significant ethanol-induced changes relative to corresponding saline baseline levels within each sex/drinking condition; (#) - significant increases in baseline levels following saline injection in high versus low drinking animals of each sex.

Figure 3.

Changes in social investigation induced by 0.5 and 0.75 g/kg ethanol (within-subject design) in adolescent males (right) and females (left) that subsequently demonstrated low and high ethanol intake under social circumstances.

(*) - significant ethanol-induced changes relative to corresponding saline baseline levels, with data collapsed across sex and drinking condition.

Figure 4.

Changes in social preference induced by 0.5 and 0.75 g/kg ethanol (within-subject design) in adolescent males (left) and females (right) that subsequently demonstrated low and high ethanol intake under social circumstances.

(*) - significant ethanol-induced changes relative to corresponding saline baseline levels within each sex/drinking condition. (#) - significant decreases in baseline levels following saline injection in high versus low drinking females; (\$) significant decreases in baseline levels following saline injection in high drinking females relative to high drinking males.

Table 1

Ethanol intake and blood ethanol content in low and high drinking adolescent males and females assessed after the last drinking session.

	Low Drinkers		High Drinkers	
	Intake (g/kg)	BEC (mg/dl)	Intake (g/kg)	BEC (mg/dl)
Male	0.65 ± 0.15	2.25 ± 0.10	1.23 ± .10	32.50 ± 7.20
Female	0.65 ± 0.14	7.37 ± 3.9	1.76 ± 0.11*	58.00 ± 5.00*

Significantly ($p < .05$) elevated intake and BECs in high drinking females relative to their male counterparts are marked with asterisks.