

Increases in anxiety-like behavior induced by acute stress are reversed by ethanol in adolescent but not adult rats

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ABSTRACT

Repeated exposure to stressors has been found to increase anxiety-like behavior in laboratory rodents, with the social anxiety induced by repeated restraint being extremely sensitive to anxiolytic effects of ethanol in both adolescent and adult rats. No studies, however, have compared social anxiogenic effects of acute stress or the capacity of ethanol to reverse this anxiety in adolescent and adult animals. Therefore, the present study was designed to investigate whether adolescent [postnatal day (P35)] Sprague–Dawley rats differ from their adult counterparts (P70) in the impact of acute restraint stress on social anxiety and in their sensitivity to the social anxiolytic effects of ethanol. Animals were restrained for 90 min, followed by examination of stress- and ethanol-induced (0, 0.25, 0.5, 0.75, and 1 g/kg) alterations in social behavior using a modified social interaction test in a familiar environment. Acute restraint stress increased anxiety, as indexed by reduced levels of social investigation at both ages, and decreased social preference among adolescents. These increases in anxiety were dramatically reversed among adolescents by acute ethanol. No anxiolytic-like effects of ethanol emerged following restraint stress in adults. The social suppression seen in response to higher doses of ethanol was reversed by restraint stress in animals of both ages. To the extent that these data are applicable to humans, the results of the present study provide some experimental evidence that stressful life events may increase the attractiveness of alcohol as an anxiolytic agent for adolescents.

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1. Introduction

The adolescent period is associated with a high significance of interactions with peers, elevated levels of social motivation, and high frequency of stressful situations (see Spear, 2000, 2007 for references and review). Adolescents spend more time interacting with peers than individuals during any other developmental period (Hartup and Stevens, 1997), with these interactions providing a significant source of positive experiences for them (Brown, 2004; Steinberg and Morris, 2001). Given the importance of interactions with peers during adolescence and the number of different stressors to which adolescents may be routinely exposed (Buchanan et al., 1992; Collins, 2001), it should not be surprising that the attractiveness of alcohol at this age may be based, at least partly, on its properties to facilitate interactions with peers and/or to produce a calming, anxiolytic action, especially under social circumstances (Beck and Treiman, 1996; Cooper et al., 2000).

Both clinical and preclinical evidence suggest that alcohol consumption can be elevated by acute and chronic stress under some circumstances (Caldwell and Riccio, 2010; Volpicelli et al., 1990),

although the relationship between alcohol use and stressful events has been shown to be quite complex (see Uhart and Wand, 2009 for references and review). It has been suggested that stress is most strongly associated with heavy drinking in adolescence, with this association becoming considerably weaker later in life (Aseltine and Gore, 2000). This may be related in part to evidence that adolescence is characterized by elevated exposure to stressful life events (Arnett, 1999), enhanced stress reactivity (Dahl and Gunnar, 2009; McCormick and Mathews, 2007; McCormick et al., 2010; Romeo, 2010), as well as age-related changes in motivational brain systems (Doremus-Fitzwater et al., 2010; Spear, 2007).

In humans, adolescence generally refers to a transitional period between youth and maturity that occurs predominantly during the second decade of life and is characterized by marked behavioral, physiological, hormonal, and neural alterations. In rats, many of these alterations are evident between postnatal days (P) 28 and 42, although some may extend, especially in males, until approximately P55 (see Spear, 2000 for references and review). During this age range adolescent rats, like their human counterparts, not only demonstrate more social behavior than younger and older individuals (Vanderschuren et al., 1997; Varlinskaya and Spear, 2008) and find these social stimuli more rewarding than adults (Douglas et al., 2004), but also ingest more ethanol on a g/kg basis than adults under various testing circumstances (Brunell and Spear, 2005; Vetter et al., 2007; Vetter-O'Hagen et al., 2009). Adolescent rodents have

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also sometimes been reported to be more responsive to stress-induced decreases in body weight (Doremus-Fitzwater et al., 2009; Stone and Quartermain, 1997) and to generally demonstrate more prolonged hormonal stress responses than adults (Brunell and Spear, 2005; Doremus-Fitzwater et al., 2009; Romeo, 2010; Romeo et al., 2006).

Repeated exposure to stressors has been found to increase anxiety-like behavior in laboratory rodents (Gehlert et al., 2005; Sajdyk et al., 2006; Sevgi et al., 2006), with the social interaction test being extremely sensitive to these anxiogenic effects (Doremus-Fitzwater et al., 2009). Our recent study has shown that levels of anxiety were not affected in adolescent and adult rats following repeated restraint when these animals were tested on an elevated plus maze; however restraint stress increased anxiety at both ages in the modified social interaction test (Doremus-Fitzwater et al., 2009). In the conventional social interaction test which is widely used for assessments of anxiogenic and anxiolytic manipulations (see File and Seth, 2003 for references and review), a pair of rats is placed into a testing chamber, and overall time spent in social interactions is used as a dependent variable (File, 1993), even though the discrete behavioral acts summed together for these assessments (e.g., following, chasing, nape attacks, pinning, sniffing) reflect behaviorally distinctive and differentially regulated forms of interactive social behaviors that include social investigation and play fighting. These two forms of social behavior have different ontogenetic patterns (Meaney and Stewart, 1981; Panksepp, 1981; Thor and Holloway, 1984; Vanderschuren et al., 1997; Varlinskaya and Spear, 2008; Varlinskaya et al., 1999) and are differentially responsive to pharmacological manipulations (see Vanderschuren et al., 1997 for references and review). For instance, play fighting has an inverted U-shaped ontogenetic pattern and peaks around P30–35, whereas social investigation is a more adult-typical form of social behavior (Panksepp, 1981; Vanderschuren et al., 1997; Varlinskaya et al., 1999). Taken together, these findings suggest that play fighting and social investigation may be mediated via different neural systems, and hence may be differentially sensitive in adolescents and adults to stress effects. Indeed, exposure to repeated restraint produced significant decreases in social investigation in both adolescents and adults, whereas play fighting was not affected by this stressor (Doremus-Fitzwater et al., 2009). Furthermore, the modified social interaction test (Varlinskaya et al., 1999, 2001) allows assessment not only of different components of social behavior but of social motivation as well, indexed via a coefficient of social preference/avoidance. Our previous studies have shown that social preference was decreased following repeated restraint (Doremus-Fitzwater et al., 2009; Varlinskaya et al., 2010). Therefore, the two measures of social activity, namely social investigation and social preference, are selectively sensitive to repeated restraint stress.

Repeated restraint not only increased anxiety in adolescents and adults, but also changed their sensitivity to the social consequences of ethanol. In general, under normal circumstances pronounced age-related differences are seen in the effects of ethanol on different forms of social activity (Varlinskaya and Spear, 2002). Specifically, adolescent rats 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 and, to a less extent, on social investigation. However, no socially activating effects of ethanol are observed in adult rats. In contrast, adults are more sensitive than adolescents to the socially suppressing and anxiogenic effects that emerged at 0.75–1.0 g/kg ethanol (Varlinskaya and Spear, 2002). Repeated restraint stress diminished age-related differences in sensitivity to the social consequences of acute ethanol by precipitating ethanol-induced social facilitation and eliminating the social inhibition in adult animals (Varlinskaya et al., 2010). Moreover, anxiety induced by repeated restraint under social test circumstances and indexed via decreases in social preference was effectively attenuated

by acute ethanol in both adolescent and adult rats (Varlinskaya et al., 2010). Such stress-associated enhancement in sensitivity to the socially facilitating as well as anxiolytic effects of ethanol may be related either to proximal effects of the final stressor exposure prior to social interactions on test day, or to chronic adaptations to the repeated stressor exposure within the neural systems modulating ethanol-induced social facilitation and anxiolysis. Indeed, reminiscent of the effects of repeated stress, a single exposure to a number of different stressors has been also reported to produce social avoidance in adult rats (Haller and Bakos, 2002). To our knowledge, however, no studies to date have compared effects of acute stress on social interactions and social consequences of ethanol in adolescent and adult laboratory rodents. Consequently, the purpose of the present study was to investigate whether adolescents differ from adults in the impact of acute restraint stress on social behavior and social preference as well as on sensitivity to the social consequences of ethanol, including its socially facilitating and anxiolytic effects.

2. Methods

2.1. Subjects

Adolescent and adult male and female Sprague–Dawley rats bred and reared in our colony at Binghamton University were used in this study. A total of 72 litters provided 360 male and female offspring to serve as experimental subjects and 360 to serve as partners. All animals were housed in a temperature-controlled (22 °C) vivarium maintained on a 14-/10-h light/dark cycle (lights on at 07:00 h) with ad libitum access to food (Purina Rat Chow, Lowell, MA) and water. Litters were culled to 10 (5 male and 5 female) pups on postnatal day (P) 1 and housed with their mothers in standard maternity cages with pine shavings as bedding material. Pups were weaned on P21 and placed into standard plastic cages together with their same-sex littermates. 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.

2.2. Experimental design

The design of the study was a 2 (age) × 3 (pre-test condition) × 5 (ethanol challenge dose) × 2 (sex) factorial, with 6 animals placed into each of the 60 experimental conditions. All animals from a given litter were assigned to the same pre-test condition. To avoid the possible confounding of litter with 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.

2.3. Pre-test conditions

On the test day, male and female adolescents (P35) and adults (P70) were randomly assigned to one of the three pre-test conditions: no manipulation, restraint stress, and social deprivation. Animals in the non-manipulated control group were left undisturbed in their home cages prior to testing. Subjects in the restraint stress group were restrained for 90 min in plastic flat-bottom restrainers (6.35 cm diameter × 15.24 cm length for adolescents and 8.57 cm diameter × 21.51 cm length for adults) in a novel holding cage. Animals in the social deprivation control group were isolated in a novel cage (standard maternity cage with pine shavings) for 90 min.

As in our previous studies (Doremus-Fitzwater et al., 2009; Varlinskaya et al., 2010), restraint was used as a stressor, since it is primarily psychological in nature and does not involve physical pain

or harm to the animal (Herman and Cullinan, 1997; Weinberg et al., 2007). Given that short-term social deprivation produces no effects on social behavior and social motivation in adolescent and adult rats (Doremus-Fitzwater et al., 2009), it was included as a pre-test condition in the experimental design in order to account for the 90-min period of social isolation and exposure to a novel environment experienced by stressed animals while in the restraint tube.

2.4. Ethanol challenge

Ethanol was administered i.p. at doses of 0, 0.25, 0.5, 0.75, and 1.0 g/kg as a 12.6% (v/v) solution in physiological saline, a relatively low concentration that induces little (if any) tissue irritation at the site of injection. Dose of ethanol was varied by altering volume rather than concentration to avoid concentration-induced differences in ethanol absorption rate (Linakis and Cunningham, 1979). Control animals were injected i.p. with isotonic saline in a volume equal to the volume of the highest dose of ethanol. Solutions were administered at room temperature. The i.p. route of ethanol administration was employed in this study, as well as in our earlier studies (Varlinskaya and Spear, 2002, 2006), because 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.

2.5. Procedure

One day before testing (P34 or P69), experimental subjects were placed individually into the social interaction apparatus for 30 min to make the testing situation familiar for them (see File, 1993 for rationale). Each test apparatus (30×20×20 cm for adolescents and 45×30×20 cm for adults) was composed of Plexiglas (Binghamton Plate Glass, Binghamton, NY) and was divided into two equally sized compartments by a clear Plexiglas partition with an aperture (7×5 cm for adolescents and 9×7 cm for adults) to allow movements of the animals between compartments.

On test day, immediately after the 90-minute pre-exposure period for the restraint stress and social deprivation groups, or following removal from the home cage for non-manipulated subjects, animals were injected with one of the 5 doses of ethanol, marked by a vertical line on the back, and placed individually in a holding cage (standard maternity cage with pine shavings) for 30 min. This pre-test social deprivation in a novel environment is a standard procedure used to increase baseline levels of social behavior (see File, 1993) from which both stimulatory and inhibitory effects of ethanol on social interactions may be readily detected (Varlinskaya and Spear, 2002, 2006). Then each experimental animal was placed into the testing apparatus and immediately exposed, for 10 min, to a non-drug-treated peer of the same age and sex. Partners were always unfamiliar with both the test apparatus and the experimental animal and were not socially deprived prior to the test (Varlinskaya and Spear, 2002, 2006, 2008). Weight differences between test subjects and their partners were minimized as much as possible, with this weight difference not exceeding 10 g at P35 or 20 g at P70, and test subjects always being heavier than their partners.

During the 10-minute test session, the behavior of the animals was recorded by a video camera (Panasonic model AF-X8, Secaucus, NJ), with real time being directly recorded onto the videotape for later scoring (Easy Reader II Recorder; Telcom Research TCG 550, Burlington, Ontario). All testing procedures were conducted between 9:00 and 13:00 h under dim light (15–20 lx). Trunk blood samples were collected immediately after the test.

2.6. Behavioral measures

The frequencies of social investigation and play fighting were analyzed from the video recordings (Meaney and Stewart, 1981; Thor

and Holloway, 1984; Vanderschuren et al., 1997; Varlinskaya and Spear, 2002, 2006; Varlinskaya et al., 1999, 2001). Social investigation was defined as the sniffing of any part of the body of the partner. 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 and Pellis, 1987). In adult animals, serious fighting is characterized by threat postures – a sideways or an upright stance with head and fore body 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 and Blanchard, 1977). In the present experiments, subjects did not demonstrate threats or serious attacks, and hence frequency of aggressive behavior (serious fighting) was not scored.

Social preference/avoidance 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). Social motivation was assessed by means of 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 (Varlinskaya et al., 1999).

In addition to the assessment of social behaviors, total number of crossovers (movements between compartments) demonstrated by each experimental subject was determined and used as an index of general locomotor activity in this social context (Varlinskaya and Spear, 2002).

Behavioral data were scored from the videotape records by trained observers without knowledge of experimental condition of any animal. Agreement between observers scoring the same videotaped interactions was in excess of 90% for each measure of social behavior.

2.7. Blood ethanol determination

For analysis of blood ethanol content (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.

2.8. Data analyses

Data for each dependent variable (social investigation, preference coefficient, play fighting, and total number of crossovers) were analyzed using separate 2 (age)×3 (pre-test condition)×5 (ethanol challenge dose)×2 (sex) analyses of variance (ANOVAs). Significant main effects of age as well as significant age×ethanol dose and age×pre-test condition interactions emerged in these overall ANOVAs for each of the behavioral measures (all p s<0.001), suggesting

pronounced age-related differences not only in ethanol sensitivity but in stress responsiveness as well. Therefore, each of the behavioral measures was examined separately at each age using 3 (pre-test condition) \times 5 (ethanol challenge dose) \times 2 (sex) ANOVAs. Where significant interactions involving pre-test condition and ethanol challenge dose were evident, planned one-way ANOVAs within each pre-test condition were conducted to explore consequences of acute stress on responsiveness to ethanol challenge. Ethanol-induced changes were assessed by post-hoc comparisons (Fisher's planned least significant difference test) between ethanol-challenged groups and saline-challenged controls.

3. Results

3.1. Social investigation frequency

Acute restraint stress influenced baseline levels of social investigation and sensitivity to the effects of ethanol among adolescent

animals [pre-test condition \times ethanol dose interaction, $F(8, 150) = 7.15$, $p < 0.0001$]. As shown in Fig. 1 (top), restraint stress, but not the same length of social deprivation, induced an anxiogenic-like reduction in baseline levels of social investigation in adolescents when these animals were compared with non-manipulated controls. This stress-induced suppression in baseline levels of social investigation was reversed by acute ethanol challenge [$F(4, 55) = 14.35$, $p < 0.0001$], with all four doses of ethanol significantly increasing social investigation in restrained adolescents (see Fig. 1, top panels). Typical ethanol-induced social facilitation was seen in non-manipulated adolescents following 0.5 g/kg ethanol, with no ethanol-induced suppression of social investigation emerging at the higher doses of ethanol in these control animals [$F(4, 55) = 4.46$, $p < 0.01$]. Pre-test social deprivation in a novel cage eliminated this stimulatory effect of ethanol and enhanced sensitivity to the suppression of social investigation that emerged at higher doses, revealing an inhibitory effect at 1.0 g/kg ethanol [$F(4, 55) = 6.84$, $p < 0.001$]. Adolescent responding to social deprivation and restraint, as well as to

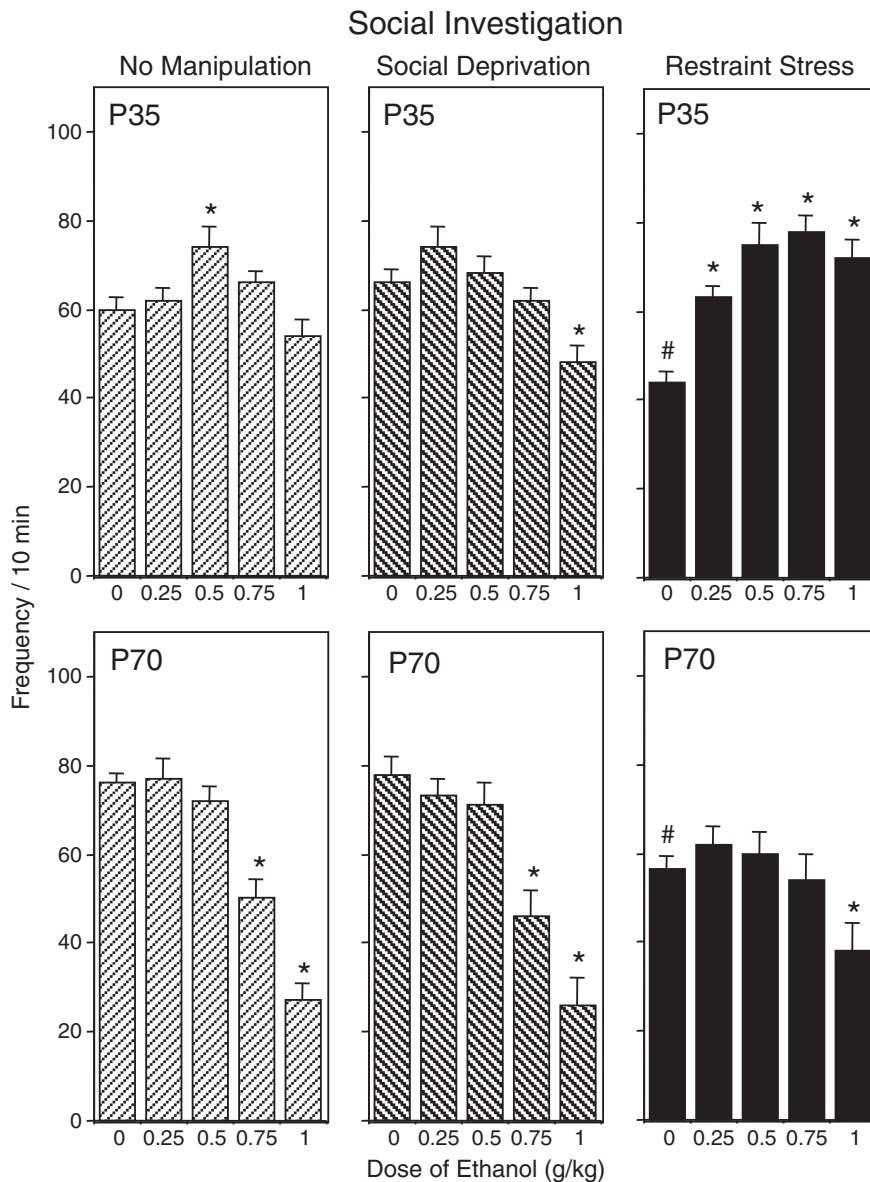


Fig. 1. The impact of acute restraint stress or social deprivation on ethanol-induced alterations in social investigation in adolescent (top panels) and adult (bottom panels) male and female rats during a 10-min social interaction test. Asterisks (*) indicate significant dose differences when compared with the corresponding saline control ($p < 0.05$), whereas pound signs (#) indicate significant reductions in social investigation relative to non-manipulated animals following acute saline challenge.

acute ethanol challenge, did not differ as a function of sex for this or any of the other behavioral measures.

The analysis of social investigation in adult animals revealed a significant pre-test condition \times ethanol dose interaction, $F(8, 150) = 3.74$, $p < 0.001$ (see Fig. 1, bottom). Similar to their adolescent counterparts, adults exposed to restraint stress, but not to social deprivation, prior to testing showed a significant reduction in social investigation when compared with non-manipulated controls following acute saline challenge. Ethanol induced a suppression of social investigation at 0.75 and 1 g/kg in non-manipulated [$F(4, 55) = 32.52$, $p < 0.0001$] and socially deprived adults [$F(4, 55) = 30.93$, $p < 0.0001$]. Acute restraint decreased sensitivity of adult animals to this ethanol-induced inhibition [$F(4, 55) = 3.63$, $p < 0.05$], with the suppression of social investigation evident at the highest ethanol dose in these acutely restrained adults. However, in marked contrast to stressed adolescents, no stimulatory, anxiolytic-like effects of ethanol on social investigation emerged in adults following acute restraint. In contrast to non-manipulated adolescents, no stimulatory effects of lower doses were evident in adults regardless of pre-exposure condition.

In adults, ethanol challenge dose also interacted with sex in the analysis of social investigation, [$F(4, 150) = 4.20$, $p < 0.01$], with adult females, regardless of pre-test condition, being less sensitive to the reductions in social investigation emerging at higher ethanol doses than their male counterparts (see Table 1).

3.2. Social preference/avoidance

In adolescents, ethanol-induced changes in social motivation, as indexed by the social preference/avoidance coefficient, differed as a function of pre-test condition [ethanol dose \times pre-test condition interaction, $F(8, 150) = 3.501$, $p < 0.001$]. Pre-exposure to restraint stress again induced an anxiogenic-like effect in adolescents, reducing baseline (i.e., at 0 g/kg) preferences for social stimuli relative to the non-manipulated group. The stress-induced attenuation in this index of social motivation was reversed by 0.5, 0.75, and 1.0 g/kg ethanol [$F(4, 55) = 4.55$, $p < 0.01$]. As seen in Fig. 2 (top), no effects of acute ethanol challenge on social preference were seen within the tested dose range in non-manipulated rats [$F(4, 55) = 0.70$, $p = 0.73$], whereas socially deprived adolescents showed a significant decrease of the coefficient at 1.0 g/kg ethanol [$F(4, 55) = 3.98$, $p < 0.01$].

The ANOVA of the preference/avoidance coefficient in adults revealed significant main effects of pre-test condition, $F(2, 150) = 6.35$, $p < 0.005$, ethanol dose, $F(4, 150) = 28.36$, $p < 0.0001$, as well as an interaction between these two variables, $F(8, 150) = 2.04$, $p < 0.05$. In contrast to adolescents, stressed adults exhibited no anxiogenic-like decreases in social motivation, nor were any anxiolytic effects of ethanol evident in these animals. Typical reductions in social preference were seen in both non-manipulated [$F(4, 55) = 19.47$, $p < 0.0001$] and socially deprived adults [$F(4, 55) = 12.13$, $p < 0.0001$] following doses of 0.75 and 1 g/kg ethanol, with stressed adults

showing this reduction only after the 1 g/kg dose [$F(4, 55) = 3.72$, $p < 0.01$].

Although not interacting with the pre-test condition, a significant sex \times ethanol dose interaction, [$F(4, 150) = 5.16$, $p < 0.001$] was observed among adults in the analysis of social preference, with females again being less sensitive to ethanol-related alterations in social preference than their male counterparts (see Table 1).

3.3. Play fighting frequency

As can be seen in Fig. 3 (top), restraint stress had no anxiogenic-like effects on play fighting in adolescents, although the effects of acute ethanol were found to vary with pre-test condition [pre-test condition \times ethanol dose interaction, $F(8, 150) = 2.61$, $p < 0.05$]. In non-manipulated adolescents, play fighting was enhanced by 0.5 g/kg and suppressed by 1.0 g/kg of ethanol [$F(4, 55) = 9.53$, $p < 0.0001$]. Social deprivation shifted this dose-response curve to the left, with the facilitation of play fighting occurring at 0.25 g/kg and inhibitory effects evident at 0.75 and 1.0 g/kg ethanol [$F(4, 55) = 12.40$, $p < 0.0001$]. Restraint stress did not change responsiveness to the activating effects of ethanol on play fighting of adolescent animals, but made them resistant to the suppressing effects of ethanol on this form of social interactions [$F(4, 55) = 3.5$, $p < 0.05$].

Adults also showed no anxiogenic-like effects of acute restraint on play fighting, although ethanol-induced suppressions of play fighting in adult animals differed as a function of pre-test condition [pre-test condition \times ethanol dose interaction, $F(8, 150) = 2.19$, $p < 0.05$]. As seen in Fig. 3 (bottom), no stimulatory effects of ethanol on play fighting of adults were seen in any of the pre-test conditions. Non-manipulated [$F(4, 55) = 20.71$, $p < 0.0001$] and socially deprived animals [$F(4, 55) = 18.46$, $p < 0.0001$] demonstrated decreased levels of play fighting after 0.75 and 1.0 g/kg of ethanol, whereas previously restrained adults were less sensitive to this suppression, showing a significant decline only at a dose of 1.0 g/kg [$F(4, 55) = 4.70$, $p < 0.01$].

3.4. Number of crossovers

Baseline levels of overall activity in the social context, indexed by total number of crossovers between the compartments, were not affected by the stressor, however ethanol-induced changes in this index of activity of adolescent animals differed as a function of pre-test condition [pre-test condition \times ethanol dose interaction, $F(8, 150) = 2.46$, $p < 0.05$]. As shown in Fig. 4 (top), although non-manipulated adolescents demonstrated a significant ethanol-induced decrease in crossovers at 1.0 g/kg [$F(4, 55) = 3.42$, $p < 0.05$], this suppressant effect was not seen following restraint stress [$F(4, 55) = 0.59$, $p = 0.67$]. Effects of ethanol on locomotor activity of socially deprived adolescents were biphasic: overall number of crossovers was enhanced following administration of 0.25 g/kg ethanol and decreased at 1.0 g/kg [$F(4, 55) = 9.2$, $p < 0.0001$].

Among adults, number of crossovers differed only as a function of ethanol dose, [$F(4, 150) = 22.11$, $p < 0.001$], with adult animals demonstrating reduction of total number of crossovers following 1.0 g/kg ethanol regardless of pre-test condition.

3.5. Blood ethanol concentration

BECs increased in a dose-dependent fashion in adolescents and adults [main effect of ethanol dose, $F(3, 120) = 1410.47$, $p < 0.0001$; $F(3, 120) = 946.24$, $p < 0.0001$, respectively: see Fig. 5]. In adults, a significant main effect of sex [$F(1, 120) = 6.38$, $p < 0.05$] was also observed, with adult females having overall slightly lower BECs (51.0 ± 4.1 mg/dl) than males (56.2 ± 3.6 mg/dl).

Table 1
Sex-related differences in sensitivity to the social consequences of ethanol among adult (P70) rats, with data collapsed across pre-test condition ($n = 12$ per group).

Ethanol dose (g/kg)	Social investigation		Social preference/avoidance	
	Male	Female	Male	Female
0	69.4 \pm 3.1	71.1 \pm 3.9	33.6 \pm 4.7	24.8 \pm 7.9
0.25	72.5 \pm 3.9	68.5 \pm 3.2	32.4 \pm 4.6	25.5 \pm 5.2
0.5	71.2 \pm 3.9	65.0 \pm 3.3	40.0 \pm 5.7	28.3 \pm 5.9
0.75	44.1 \pm 4.9 *	55.7 \pm 2.8	4.1 \pm 8.5 *	19.2 \pm 6.3
1.0	22.6 \pm 1.9 *	38.1 \pm 4.6 *	-31.8 \pm 6.2 *	-5.2 \pm 6.1 *

Asterisks (*) indicate significant differences from corresponding saline controls within each sex.

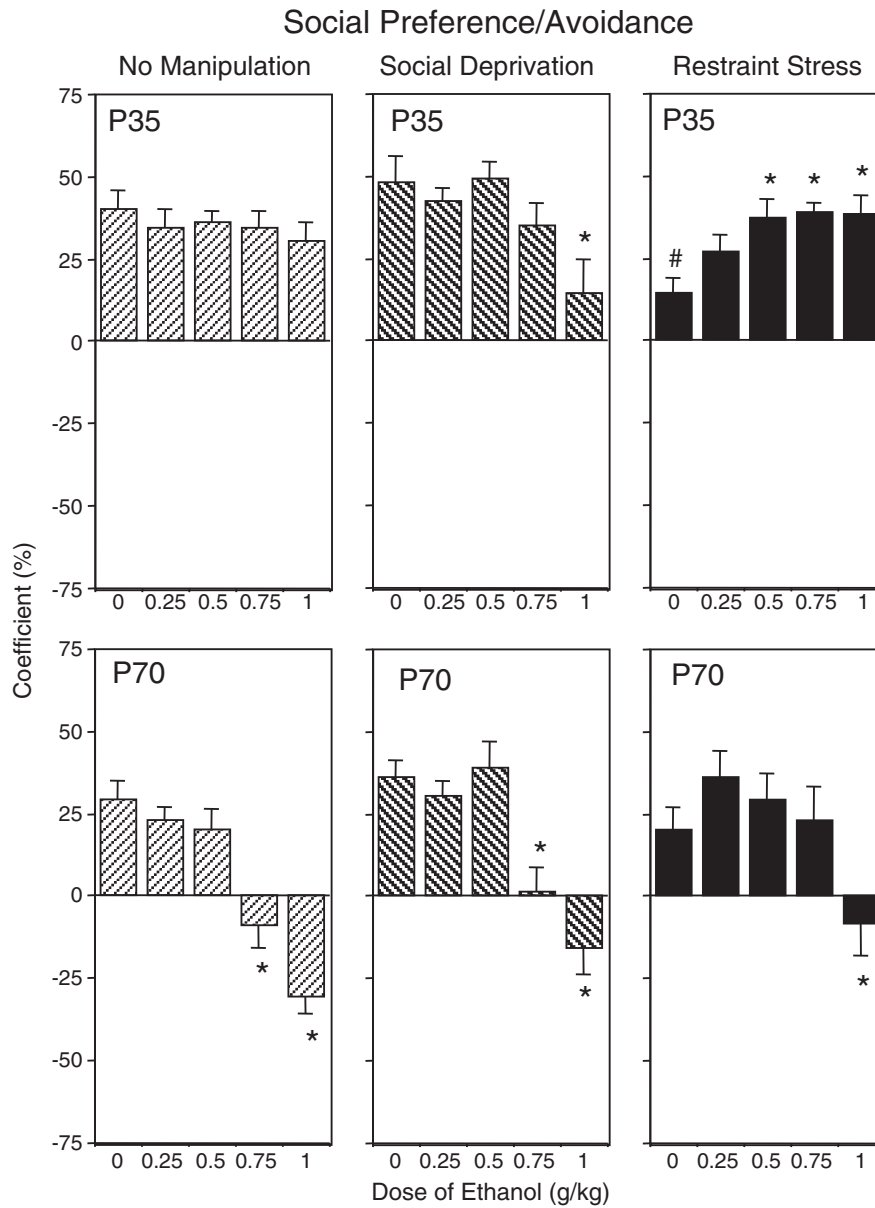


Fig. 2. The impact of acute restraint stress or social deprivation on ethanol-induced alterations in coefficients of preference/avoidance in adolescent (top panels) and adult (bottom panels) male and female rats during a 10-min social interaction test. Asterisks (*) indicate significant dose differences when compared with the corresponding saline control ($p < 0.05$), whereas pound signs (#) indicate significant reductions in the coefficient relative to non-manipulated animals following acute saline challenge.

4. Discussion

Findings of the present study demonstrate that adolescent rats differ from adults in their responsiveness to acute restraint stress in terms of both stress-induced changes in social behavior and stress-associated alterations in sensitivity to the social consequences of ethanol. Acute restraint stress increased anxiety in a social context at both ages; this anxiogenic effect was more prominent in adolescents than adults, and was suppressed by ethanol only among the adolescents. Acute restraint stress was also found to attenuate the social suppressing effects of ethanol, with this effect being more pronounced in adults than adolescents. In contrast to these sobering effects of acute restraint seen at both ages, pre-test social deprivation induced a leftward shift in the ethanol dose–response curve in adolescents, with socially deprived adolescents showing both social facilitation as well as social suppression at lower doses than adolescents who were not socially deprived. These changes in sensitivity

to ethanol following acute restraint and social deprivation are unlikely to be attributable to alterations in ethanol pharmacokinetics, given that BECs did not differ as a function of pre-test stress condition in adolescents or adults.

4.1. Behavioral effects of acute stress

Acute restraint stress increased anxiety, as indexed by reduced baseline levels of social investigation at both ages. These findings are in agreement with studies that have reported increases in anxiety-like behavior following even a single exposure to a stressor in adult animals (Gameiro et al., 2006; Haller and Bakos, 2002; Haller et al., 2003). However, when baseline levels of social preference were assessed using the preference/avoidance coefficient, age differences in responsiveness to acute stress became apparent, with adolescents but not adults showing the restraint stress-induced reduction in social preference. This finding suggests that adolescent animals may

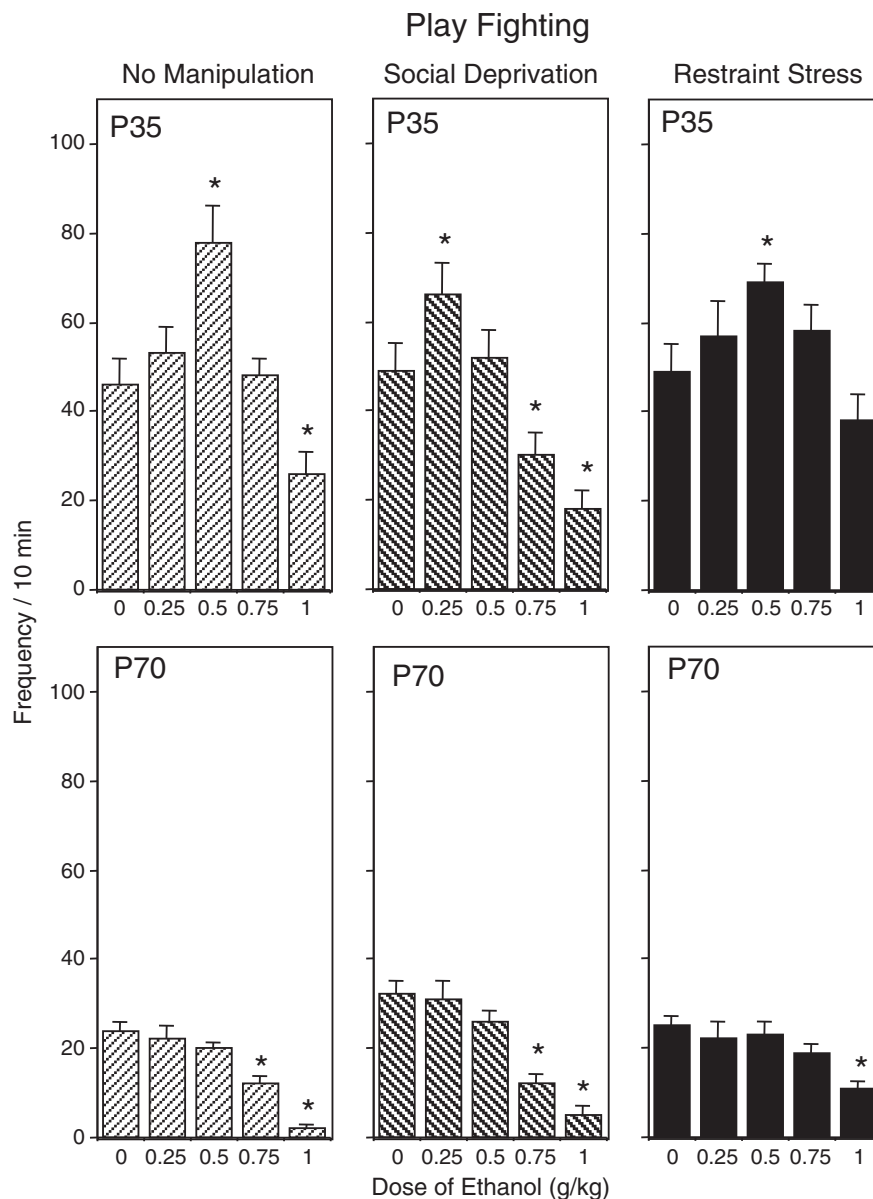


Fig. 3. The impact of acute restraint stress or social deprivation on ethanol-induced alterations in play fighting in adolescent (top panels) and adult (bottom panels) male and female rats during a 10-min social interaction test. Asterisks (*) indicate significant dose differences when compared with the corresponding saline control ($p < 0.05$).

be more sensitive than adults to the anxiogenic effects of acute restraint. Age-related differences in responsiveness to acute restraint observed in the present study are reminiscent of findings of [Romeo et al. \(2006\)](#), who reported greater PVN activation, indexed via Fos expression, in adolescents than adults following acute restraint stress.

These anxiogenic effects of acute restraint were specific to social investigation and social preference, with baseline levels of play behavior and overall locomotor activity being unaffected by acute restraint at either age. These findings are consistent with our earlier work with repeated restraint ([Doremus-Fitzwater et al., 2009](#); [Varlinskaya et al., 2010](#)) and provide further support for the suggestion that play and overall locomotor activity are separable from the anxiety-like response revealed via decreases in social investigation and social preference in this test.

At both ages, pre-test social deprivation exerted no effects on baseline levels of social investigation, social preference, play fighting and locomotor activity. The lack of behavioral changes in this control group suggests that the anxiogenic effects of acute restraint are attributable in part to the restraint process per se rather than merely

a function of 90 min of social isolation in a novel environment. Repeated social deprivation (90 min/day for 5 days), however, was previously found to increase play fighting in adolescents and adults, although again no anxiogenic effects (indexed via decreases in social investigation and/or social preference) were seen ([Doremus-Fitzwater et al., 2009](#)). Taken together, these observations suggest that relatively brief social deprivations differ dramatically from comparable amounts of restraint stress in terms of social consequences in both adolescents and adults. These findings are similar to prior work conducted primarily in adults showing that different stressors produce differential behavioral changes ([Mercier et al., 2003](#)) and neural alterations, including brain activation patterns indexed by Fos expression ([Dayas et al., 2001](#)) and corticosterone response ([Bowers et al., 2008](#)).

4.2. Stress-induced changes in ethanol sensitivity

Acute restraint not only produced behavioral alterations, but also changed sensitivity to the social consequences of ethanol. Specifically,

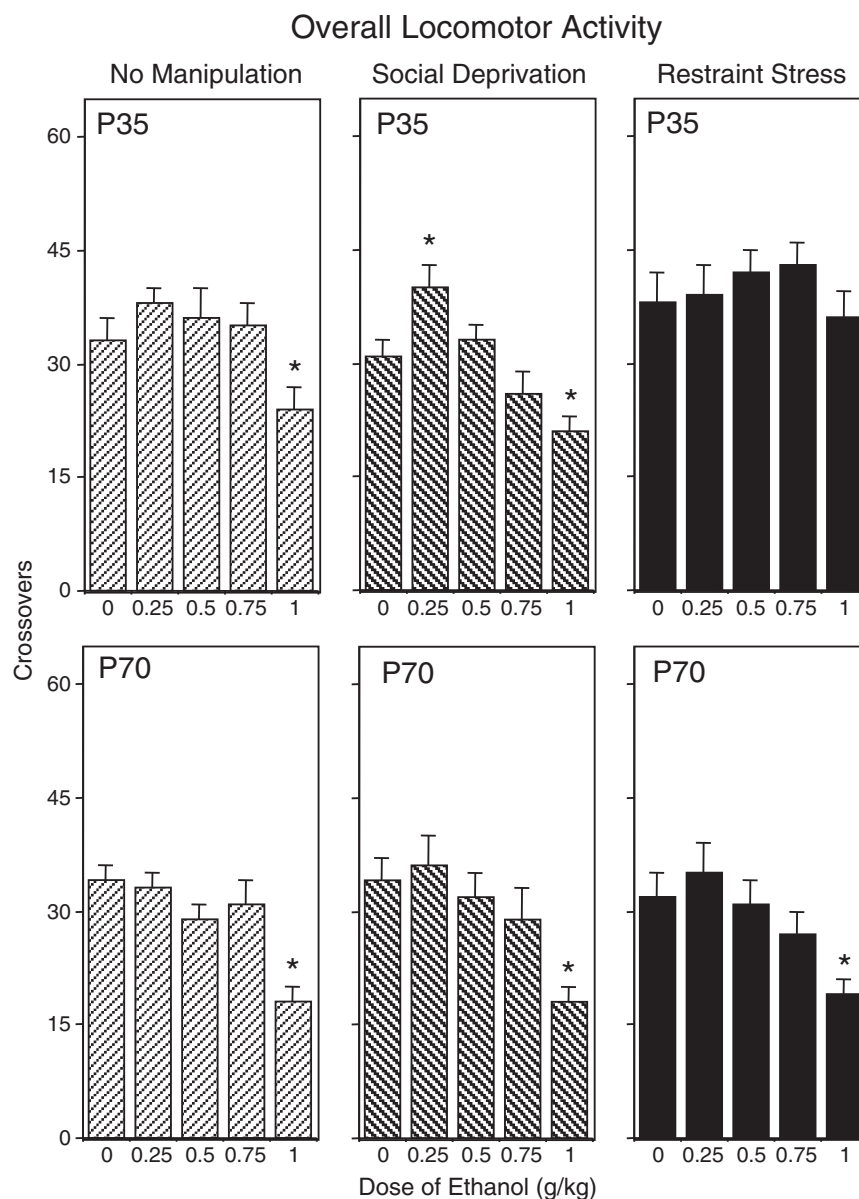


Fig. 4. The impact of acute restraint stress or social deprivation on ethanol-induced alterations in number of crossovers exhibited by adolescent (top panels) and adult (bottom panels) male and female rats during a 10-min social interaction test. Asterisks (*) indicate significant dose differences when compared with the corresponding saline control ($p < 0.05$).

the behavioral effects of acute restraint stress in adolescent animals, indexed by reduced baseline levels of social investigation and social preference, were reversed by acute ethanol challenge, with all test doses of ethanol significantly increasing social investigation and the three highest doses enhancing social preference in restraint stress-exposed adolescent rats. In contrast, ethanol was ineffective in mitigating anxiogenic-like effects of acute restraint in adults. This result was rather unexpected, given that adult rats have previously been reported to become more sensitive to anxiolytic drugs following a single exposure to different stressors (Haller et al., 2003), although anxiolytic effects of ethanol were not tested in this study.

Whereas acute restraint had no impact on the low-dose facilitation of play fighting induced by ethanol among adolescents in the present study, this acute stressor did decrease adolescent sensitivity to the ethanol-induced suppression of play fighting and locomotor activity emerging at higher doses of ethanol. Among adults, acute restraint was even more effective in attenuating sensitivity to the suppressing effects of ethanol, an effect seen with all indices of social activity (but not locomotor activity). Thus, acute restraint had an

apparent sobering effect at both ages when indexed via attenuations in the social inhibitory effects of ethanol. Similar sobering effects of acute restraint have been reported in adult inbred long-sleep mice, with these mice demonstrating reduced sensitivity to ethanol-induced sedation following a 30-min period of acute restraint, as indexed by a decrease in the duration of loss of the righting reflex and, most importantly, an increase in BEC at regain of the righting response (Parker et al., 2008).

In contrast to these sobering effects of acute restraint observed in adolescents and adults, pre-test social deprivation conversely increased sensitivity of adolescents to the socially suppressing effects of ethanol, as well as to the activating effects of ethanol on play fighting and overall motor activity in the social context, therefore producing a leftward shift in ethanol sensitivity among adolescents tested in a social context. These effects, however, were age-specific, with pre-test social deprivation having no impact on the sensitivity of adults to the social consequences of ethanol. Similarly to the apparent leftward shift in ethanol sensitivity seen in adolescents following pre-test social deprivation, in other work we have found adolescent

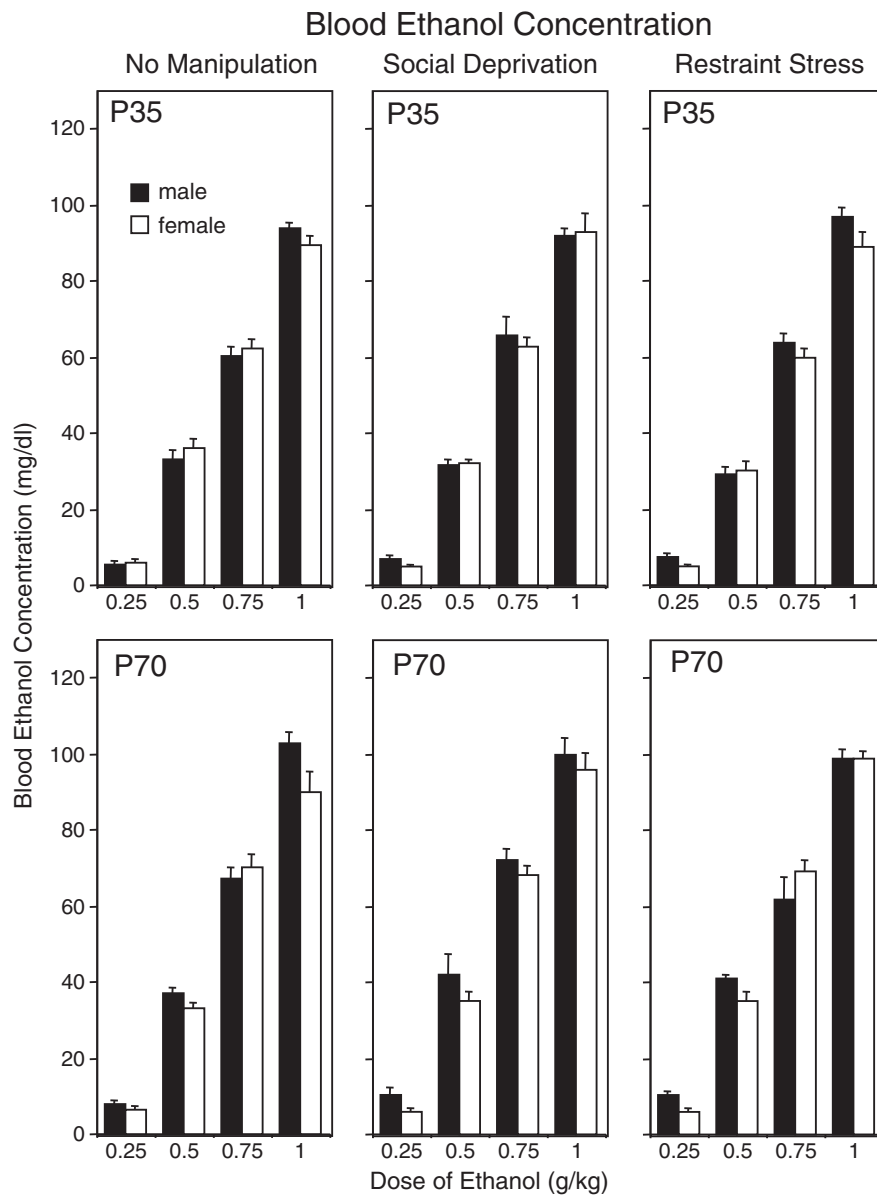


Fig. 5. Blood ethanol concentrations following acute challenge with 1 of 4 doses of ethanol assessed immediately after social testing in adolescent (top panels) and adult (bottom panels) male and female rats.

males to be more sensitive to the aversive effects of ethanol indexed via a conditioned taste aversion paradigm when animals were socially deprived during intoxication relative to those who experienced ethanol intoxication in the presence of a peer (Vetter-O'Hagen et al., 2009). These studies hint to a potentially important role for social context in influencing ethanol sensitivity, although the absence of other work in this area precludes further discussion of these effects at present.

4.3. Similarities and differences in the effects of acute versus repeated restraint

Both similarities and differences were seen between consequences of acute restraint assessed in the present study and prior studies involving repeated restraint. Behaviorally, the anxiogenic effects of acute restraint in adolescent and adult rats were similar to those of repeated exposure to this stressor (Doremus-Fitzwater et al., 2009), with both acute and repeated restraint decreasing social investigation and social preference (although the latter was restricted

to acutely restrained adolescents in the present study). Similarities also emerged between consequences of acute and repeated restraint in terms of ethanol-related sobering effects, with repeated restraint, like the acute restraint stressor used in the present study, decreasing sensitivity to the socially suppressing effects of ethanol in both adolescents and adults (Varlinskaya et al., 2010). Acute and repeated restraint also has been shown to decrease sensitivity to the sedative-hypnotic effects of ethanol in adult inbred long-sleep mice (Parker et al., 2008). These sobering effects of stress are not restricted to laboratory rodents, but have been reported in humans as well (Breslin et al., 1994, 1995).

Yet, some differences between the consequences of acute and repeated restraint also were apparent, effects particularly dramatic in terms of play fighting. Whereas acute restraint had no impact on ethanol-induced facilitation of play fighting among adolescent animals in the present study, exposure to repeated restraint not only increased sensitivity of adolescents to the stimulatory effects of ethanol on play fighting, but also precipitated the expression of ethanol-induced social facilitation among adults (Varlinskaya et al., 2010).

Taken together, these results suggest that the neural substrates of play fighting implicated in the stimulatory effects of ethanol were not affected by acute restraint.

The enhancement of ethanol-induced anxiolysis by acute restraint in adolescents but not adults, as seen here, differs substantially from the enhancement of the anxiolytic effects of ethanol previously observed following repeated restraint. Exposure to repeated restraint stress suppressed baseline levels of social investigation and social motivation in both adolescents and adults (Doremus-Fitzwater et al., 2009), with these anxiogenic consequences of repeated restraint on social preference, but not on social investigation, reversed by acute ethanol regardless of age (Varlinskaya et al., 2010). This pattern of results suggests that enhanced sensitivity to the anxiolytic effects of ethanol in stressed adolescents may be related to immediate stress-induced alterations in the neural substrates of anxiety (Brandão et al., 2003; Shekhar et al., 2005). In adults, however, delayed adaptations to a repeated stressor may play a substantial role in the enhancement of responsiveness to ethanol-associated anxiolysis.

4.4. A new model of social anxiety?

In humans, social anxiety is associated with alcohol use (Buckner et al., 2006; Burke and Stephens, 1999). 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). 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 effects of alcohol on social anxiety are still limited (see Carrigan and Randall, 2003 for references and review), especially among underage youth where ethical considerations obviate direct assessment of the socially anxiolytic effects of alcohol. Therefore, animal models that will allow assessments of anxiolytic effects of ethanol among socially anxious adolescents and determination of relationships between social anxiety and drinking during adolescence are of particular importance.

Anxiolytic effects of ethanol can be assessed in a number of different paradigms, including assessment of social interactions in an unfamiliar and/or brightly lit environment. In general, an unfamiliar environment is viewed as an anxiety-provoking situation, with the decreases in social interactions seen under this condition used as an experimental model of generalized anxiety (File, 1980) induced by manipulations of the environment that generate uncertainty (unfamiliarity) or fear (bright light). Ethanol-induced anxiolysis has been reported in both adolescents and adults under these environment-related anxiogenic test circumstances, although adolescents have been found to be less sensitive to the anxiolytic effects of ethanol than adults under these test circumstances (Varlinskaya and Spear, 2002). These findings contrast markedly with the anxiety induced by acute restraint stress when animals were tested in a familiar and hence non-anxiogenic environment, with restraint stress pre-exposure dramatically enhancing sensitivity of adolescent (but not adult) animals to the anxiolytic effects of ethanol.

Given these dramatic differences in adolescent sensitivity to the anxiolytic effects of ethanol (i.e., insensitivity under unfamiliar test circumstances versus enhanced sensitivity in the familiar environment following restraint stress), it is possible that the anxiety provoked by unfamiliar test situations may differ from that induced by acute (present study) or repeated (Varlinskaya et al., 2010) restraint stress, with the former modeling generalized anxiety (File, 1980), but the later, perhaps, providing a model of social anxiety. Indeed, the modified social interaction test in a familiar, non-anxiogenic environment, by allowing assessments not only of different components

of social behavior but also social motivation (indexed via a coefficient of social preference/avoidance), shows extreme sensitivity to stress-induced behavioral changes, with selective stress-related decreases in social investigation and social preference that were likewise selectively sensitive to the anxiolytic effects of ethanol in stressed animals (Varlinskaya et al., 2010). These observed changes in social responsiveness and responsiveness to the anxiolytic effects of ethanol following acute and repeated restraint may prove useful as a model of stress-induced social anxiety in the rat, with adolescents being particularly vulnerable to this social anxiety associated with stress. Clearly though, more studies are necessary to behaviorally and pharmacologically validate this new model and to assess its face and construct validity.

Overall, the results of the present study show that effects of acute restraint stress on ethanol sensitivity differ as a function of age: the anxiogenic-like effects of restraint stress were reversed by ethanol only in adolescents, whereas sobering effects of restraint stress in terms of attenuating the socially suppressing effects of higher doses of ethanol were evident at both ages, but were particularly pronounced in adults. To the extent that these data are applicable to humans, the results of the present study provide some experimental evidence that acute stressful experiences, producing social anxiety, may markedly increase the attractiveness of alcohol as a socially anxiolytic agent for adolescents, whereas sobering effects of acute stressors may permit enhanced alcohol consumption in social settings without the emergence of socially suppressing effects that could serve as cues to temper or terminate drinking episodes.

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