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Ontogeny of Acute Tolerance to Ethanol-Induced Social Inhibition in Sprague–Dawley Rats

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Abstract

Background— Adolescent rats are less sensitive than adults to a number of acute effects of ethanol, including ethanol-induced social inhibition. Adolescent insensitivity to the suppressing effects of ethanol on social interactions could be related in part to age differences in compensatory responses, including acute tolerance, that serve to counteract these inhibitory effects of ethanol. The present study explored ontogenetic development of acute tolerance within 30 minutes after administration of a relatively low ethanol dose, using ethanol-induced social impairment as the target response measure.

Methods— Overall social activity was examined following challenge with 1 g/kg ethanol (intraperitoneally) at 2 postinjection intervals (5 or 30 minutes) in early [postnatal day (P) 28], mid (P35), or late (P42) adolescent or adult (P70) group-housed male and female Sprague–Dawley rats (Experiment 1). Blood and brain ethanol concentrations (BECs and BrECs) were assessed in separate groups of animals 5 or 30 minutes after ethanol administration (Experiment 2). Expression of acute tolerance was examined by assessing the relationship between BrECs and the degree of social impairment in individual animals at P28, P35, P42, and P70 during early recovery period (up to 30 minutes) following acute ethanol challenge (Experiment 3).

Results— Effects of ethanol on overall social activity were age-dependent and time-dependent. Whereas all age groups showed equivalent ethanol-induced social inhibition 5 minutes after injection, testing at 30 minutes revealed marked age differences. Social inhibition was still pronounced at this time in adults, but was diminished in an age-related manner at younger ages (Experiment 1). In contrast to the ontogenetic differences in rates of decline in social impairment across time, decreases in brain and blood ethanol levels over time were similar across age (Experiment 2). Only P28 and P35 adolescents showed acute tolerance to ethanol-induced social inhibition, as indexed by an increasing time-dependent dissociation between BrECs and ethanol-induced social impairment, with social impairment declining faster than BrECs (Experiment 3).

Conclusions— This is the first study to document enhanced acute tolerance in adolescent rats relative to adult animals at nonhypnotic doses of ethanol. The greater expression of acute tolerance in young animals may reflect an enhanced predisposition of their nervous systems to respond rapidly to even modest doses of ethanol with compensatory adaptations. A greater propensity of early adolescents to develop acute tolerance may contribute to their resistance to adverse effects of ethanol, thereby permitting heavy drinking at this age and placing early adolescents at higher risk for extensive alcohol use.

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Keywords

Adolescence; Ethanol; Acute Tolerance; Social Behavior; Rat

Unique adolescent-specific neural alterations may influence responsiveness to ethanol among adolescents in a way that differs from adult responding (Spear, 2000a,2000b;Witt, 1994). And, indeed, there is convincing experimental evidence that responsiveness to ethanol changes across ontogeny, although these ontogenetic patterns of ethanol responsivity vary with the effect of ethanol being examined. For instance, adolescent rats appear to be more sensitive than adults to ethanol-induced impairments in hippocampal long-term potentiation (Pyapali et al., 1999;Swartzwelder et al., 1995a,1995b) and spatial memory (Markwiese et al., 1998), as well as to the facilitation in social behavior that is seen following challenge with low doses of ethanol (Varlinskaya and Spear, 2002). On the other hand, when compared with their adult counterparts, adolescent rats are less sensitive to a variety of acute effects of ethanol, including ethanol-induced motor impairment (Hollstedt et al., 1980;Silveri and Spear, 1998,2001;White et al., 2002), sedation (Little et al., 1996;Moy et al., 1998), and anxiolysis (Varlinskaya and Spear, 2002). Sensitivity to hypothermic effects of ethanol has been also reported to increase across ontogeny (Silveri and Spear, 2000), although findings with this measure are more mixed (Brasser and Spear, 2002;Ristuccia and Spear, 2005;Swartzwelder et al., 1998) and may be related to the amount of perturbation of the test circumstances (Ristuccia and Spear, under revision).

Among the ethanol consequences to which adolescent animals appear to be less sensitive than adults is ethanol-induced social inhibition. When tested for social interactions in a familiar (low-anxiety producing) environment 30 minutes after challenge with moderate doses of ethanol, adolescent rats at postnatal day (P) 35 showed less social inhibition than their adult (P70) counterparts (Varlinskaya and Spear, 2002). It seems unlikely that this adolescent-associated attenuation in sensitivity to the suppressing effects of ethanol on social interactions is a function of ontogenetic differences in ethanol pharmacokinetics, given that no age-related differences in blood ethanol concentrations (BECs) were found 30 minutes after the ethanol challenge doses. Interestingly, ontogenetic increases in responsiveness to the inhibitory effects of ethanol on social behavior are apparent even across the adolescent period, with early (P28) adolescents being less sensitive to ethanol-induced social inhibition than mid (P35) and late (P42) adolescents (Varlinskaya and Spear, 2004).

A relative insensitivity of adolescent rats to ethanol-induced social inhibition could be related in part to age differences in compensatory responses, including acute tolerance, that serve to counteract these inhibitory effects of ethanol. Acute tolerance, defined as a reduced sensitivity to ethanol that emerges within a single exposure, is traditionally characterized by less impairment at the same BEC on the descending than the ascending portion of the BEC time curve (Mellanby, 1919) or by higher BECs or brain ethanol concentrations (BrECs) at the offset than at the onset of a particular behavioral or physiological impairment (Lê et al., 1992). Acute tolerance has been observed for a number of ethanol effects, including motor impairment, sedation, and hypothermia in adult rodents (see Lê and Mayer, 1996, for references and review). Studies examining the ontogeny of acute tolerance have been restricted to the assessments of ethanol-induced sedation emerging at relatively high doses of ethanol and have reported ontogenetic declines in acute tolerance to the hypnotic effects of ethanol, with more pronounced acute tolerance expressed in adolescent than adult rodents (Draski et al., 2001;Grieve and Littleton, 1979;Silveri and Spear, 1998,2002). Ontogenetic alterations in the development of acute tolerance to other ethanol effects have not been explored.

These initial assessments reporting an ontogenetic decline in acute tolerance focused on ethanol's sedative effects at relatively high doses (from 2.5 to 5 g/kg). It is not yet known whether similar ontogenetic differences in acute tolerance would be seen at lower doses, more typical of those associated with human ethanol consumption levels (see Eckardt et al., 1998, for references and review). In contrast to the relatively high doses required for the assessment of ethanol-induced sedation, the social interaction test is sensitive to acute effects of low-to-moderate doses of ethanol. With this test, social impairment can be detected at doses of 1 g/kg in adolescents and even less in adults (Varlinskaya and Spear, 2002), doses in the range of those used in studies of acute tolerance in human volunteers (e.g., Bennett et al., 1993; Hiltunen, 1997a, 1997b). Therefore, the social interaction test shows promise as a tool for assessing the development of acute tolerance to social inhibition induced by moderate doses of ethanol in adolescent and adult animals.

Acute tolerance can be assessed in a number of ways, many of which have focused on the relationship between BECs and ethanol-induced impairment of a certain function during the descending portion of the blood alcohol curve. For instance, to the extent that acute tolerance is a linear process with time, this tolerance can be indexed by the increasing dissociation between BEC and ethanol-induced impairment following a given dose of ethanol, with ethanol-induced impairment declining more rapidly than BECs (Khanna et al., 2002; Radlow, 1994). There is experimental evidence, however, that acute tolerance to some ethanol effects emerges extremely rapidly and may be seen within minutes following ethanol administration in both electrophysiological (Palmer et al., 1992; Pearson et al., 1997; Sorensen et al., 1980) and behavioral studies (Deitrich et al., 2000; Gill and Deitrich, 1998). This rapid emergence of acute tolerance can be detected in animals given a single ethanol dose by examining the association between the incidence of the target behavior and BEC beginning shortly following injection. For instance, with the later approach, acute tolerance has been assessed by testing animals at 1-minute intervals for the first 5 minutes following ethanol administration and then at 5-minute intervals thereafter for a total of 30 minutes (Deitrich et al., 2000; Gill and Deitrich, 1998), thus examining animals during the ascending, peak, and early descending portions of the blood curve. Using this paradigm, some strains of mice were observed to develop tolerance to the ataxic effects of ethanol measured on the rotorod within 10 minutes following a single ethanol injection (Gill and Deitrich, 1998).

Given that previous studies reporting ontogenetic declines in the development of acute tolerance used relatively high doses of ethanol and assessed emergence of tolerance to ethanol-induced sedation over a period of several hours (Draski et al., 2001; Grieve and Littleton, 1979; Silveri and Spear, 1998, 2002), the main objective of the present study was to examine ontogenetic development of acute tolerance to a relatively low dose of ethanol (1 g/kg) when examined within 30 minutes after ethanol administration, using ethanol-induced social impairment as the target response measure. Because of recent findings (e.g., Philpot et al., 2003; Varlinskaya and Spear, 2004) that point to age differences in ethanol responsiveness even within the adolescent period, rats tested at a variety of ages (P28, P35, P42) in adolescence were compared with young adult (P70) animals.

GENERAL METHODS

Subjects

Sprague-Dawley rats bred and reared in our colony at Binghamton University were used in these experiments. All animals were housed in a temperature-controlled (22 °C) vivarium maintained on a 14-hour/10-hour light/dark cycle (lights on at 07:00 AM) with ad libitum access to food (Purina Rat Chow, Lowell, MA) and water. Pups were housed until weaning with their mothers in standard maternity cages with pine shavings as bedding material. Litters were culled to 10 (5 males and 5 females) pups within 24 hours after birth on PO. Rats were

weaned on P21 and placed into standard plastic cages with same-sex littermates (5 animals in a cage). Animals were tested either on P28 (early adolescents), P35 (mid adolescents), P42 (late adolescents), or P70 (young adults). 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.

Drug Challenge

Ethanol was administered intraperitoneally (i.p.) as a 12.6% (v/v) solution in physiological saline at a dose of 1 g/kg. Control animals were injected with isotonic saline in a volume equal to the volume of ethanol administered (1% body weight). Solutions were administered at room temperature.

Social Interaction Test

One day before testing (P27, P34, or P41 for adolescents and P69 for adults), all experimental animals were placed individually into the testing chamber for 30 minutes to make the experimental situation familiar for them. The test apparatuses consisted of Plexiglas (Binghamton Plate Glass, Binghamton, NY) chambers (30×20×20 cm for adolescents and 45×30×20 cm for adults) containing clean pine shavings. Each test apparatus was divided along the long axis into 2 equally sized compartments by a clear Plexiglas partition that contained an aperture (7 cm×5 cm for adolescents and 9 cm×7 cm for adults) to allow movement of the animals between compartments (Varlinskaya et al., 1999,2001).

All testing procedures were conducted between 9:00 AM and 1:00 PM under dim light (15–20 lx). Before testing, each experimental subject was placed in an individual holding cage for 30 minutes, given that pretest social deprivation has been shown to increase baseline levels of social behavior from which inhibitory effects of ethanol (i.e., social inhibition) may be more readily detected (File, 1993). Each animal was placed into the testing chamber simultaneously with a same age and sex test partner. Partners were always non-ethanol-exposed animals that had not been socially isolated before testing and who were unfamiliar with both the test apparatus and the experimental animal with which they were paired for testing. Weight differences between test subjects and their partners were minimized as much as possible. The order of testing was counterbalanced for all treatment groups. 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, ON, Canada). After each test, the apparatus was wiped with 3% peroxide hydrochloride and the shavings were replaced with fresh ones.

Behavioral Measures

The frequency of a number of social activities from the ethogram of each test subject was analyzed from the video recordings. Overall social activity was scored as the sum of the frequencies of the following social behaviors: social investigation (sniffing of any part of the body of the partner), contact (crawling over and under the partner and social grooming), and play fighting (pouncing or playful nape attack, chasing, and pinning) (Meaney and Stewart, 1981; Thor and Holloway, 1984; Vanderschuren et al., 1997). 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 forebody 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. 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). Locomotor activity was scored and analyzed to assess whether the suppressant effects of ethanol on social behavior and an emergence of acute tolerance to these effects were independent from or associated with general sedative effects of ethanol (File, 1993).

Behavioral data were scored from the videotape records by 2 observers without knowledge of the drug treatment condition of any animal using a real-time event-recording program on a Macintosh computer. Agreement between observers scoring the same videotape was in excess of 90% for each measure of social behavior.

Experimental Design and Data Analysis

Animals were tested as early (P28), mid (P35), or late (P42) adolescents or as adults (P70). Animals were assigned semirandomly to the experimental groups. Constraints with regard to group assignment were that no more than 1 subject from a given litter be assigned to a particular treatment condition to avoid the possible confounding of litter with treatment effects (Holson and Pearce, 1992), with equal number of males and females placed in each treatment group to allow analysis of sex effects.

Statistical comparisons were made using analyses of variance (ANOVAs). Tukey–Kramer tests were used to explore significant main effects and interactions. A significance level of $p < 0.05$ was used for all analyses and comparisons.

Experiment 1. Effects of Ethanol on Social Interactions of Adolescent and Adult Rats Tested 5 or 30 Minutes After Injection

In our previous experiments, age-related differences in ethanol-induced social inhibition were evident as early as 30 minutes after ethanol administration (Varlinskaya and Spear, 2002, 2004). To the extent that these age-related differences in ethanol sensitivity are influenced at least in part by ontogenetic differences in expression of acute tolerance, it would be expected that acute tolerance to the inhibitory effects of ethanol on social activity might emerge rapidly, especially in adolescent animals. If age differences in responsiveness to ethanol-induced social inhibition apparent at 30 minutes after ethanol administration were indeed related to age differences in expression of within session tolerance, differential sensitivity to the inhibitory effects of ethanol on social behavior should be less apparent at shorter injection-test intervals.

Experiment 1 explored this hypothesis by comparing ethanol-induced inhibition of social interactions at 2 postinjection intervals (5 and 30 minutes) in adolescent and adult rats after a challenge dose of 1 g/kg ethanol.

Methods—A total of 40 litters provided 160 male and female off-spring to serve as experimental subjects and 160 to serve as partners. Subjects were given either 0 or 1 g/kg ethanol and were examined at P28, P35, P42, or P70 either 5 or 30 minutes after administration, with equal numbers of males and females placed into each treatment and age group. Animals in the 30-minute time interval group were injected with ethanol or saline and then placed individually into holding cages, whereas subjects in the 5-minute group were placed into individual cages for 25 minutes, injected, and returned to individual holding cages for 5 more minutes. The design of the study was a 4 (age) × 2 (treatment) × 2 (time) × 2 (sex) factorial, with 5 experimental animals being tested in each of the 32 groups defined by this factorial design.

Overall social activity (sum of the frequencies of social investigation, contact behavior, and play) and locomotor activity (number of crossovers between the compartments) were scored and analyzed using separate 4 (age) $\times$ 2 (treatment) $\times$ 2 (time) $\times$ 2 (sex) ANOVAs. For further analysis, raw experimental data were transformed into relative measures of social impairment and impairment of locomotor activity induced by ethanol. For this purpose, the percentage decline in overall social activity (and locomotor activity, as indexed by crossovers between the compartments) after the 1 g/kg ethanol test dose was calculated relative to the corresponding saline control group for each age/sex combination at each time point. With this transformation, the maximal possible social impairment or impairment of overall locomotor activity would be 100%, whereas a value of 0% would reflect no impairment. The transformation of the raw data eliminated possible age-related differences in social and locomotor activity of saline controls, hence permitting more direct comparisons among ethanol-treated animals of different ages. Impairments of social and locomotor activity were analyzed using separate 4 (age) $\times$ 2 (time) $\times$ 2 (sex) ANOVAs.

Results

Overall Social Activity—Analysis of overall social activity revealed significant main effects of age [$F(3, 128)=11.78, p<0.001$], treatment [$F(1, 128)=227.75, p<0.001$], and time [$F(1, 128)=37.43, p<0.001$], as well as a significant 3-way interaction of these 3 variables [$F(4, 280)=3.04, p<0.05$] (see Fig. 1, top panels). Ethanol significantly decreased overall social activity in P28, P35, P42, and P70 animals tested 5 minutes after administration of the dose of 1 g/kg. However, levels of social activity of P28 animals treated with ethanol did not differ significantly from those of saline controls when testing occurred 30 minutes after administration. P35, P42, and P70 rats still demonstrated a significant decrease in overall levels of social activity at this time point.

As seen in Fig. 1, overall social activity of saline-treated animals differed slightly but significantly as a function of age and postinjection time interval. And indeed, a 4 (age) $\times$ 2 (time) $\times$ 2 (sex) ANOVA comparison of social activity in saline-treated animals revealed significant main effects of age [$F(3, 64)=3.16, p<0.05$] and time [$F(1, 64)=7.30, p<0.01$], with early adolescents showing more social activity than adults and animals generally exhibiting more social behavior 30 minutes after injection than 5 minutes after injection. Given these age-related and time-related differences in overall levels of social behavior in the saline controls, ethanol-induced social impairment was expressed as percent decrease in social activity relative to the corresponding saline control group before further analyses (see “Methods”).

Social Impairment—Social impairment decreased across the 2 postinjection intervals [a significant main effect of time, $F(1, 64)=38.09, p<0.001$], but these changes were age dependent [age $\times$ time interaction, $F(3, 64)=8.58, p<0.001$]. No significant differences across age were observed in the levels of social impairment at 5 minutes after injection, whereas P28 rats were significantly less impaired than P35, P42, and P70 animals when tested 30 minutes after ethanol administration (see Fig. 2, left), and mid adolescents (P35) were less impaired than late adolescents (P42) and adults (P70). Only early and mid adolescent rats demonstrated a significant reduction of social impairment over time.

Locomotor Activity—Ethanol at the test dose of 1 g/kg decreased locomotor activity regardless of age and postinjection interval. Analysis of total number of crossovers between the compartments revealed only main effects of age [$F(3, 128)=20.52, p<0.001$], treatment [$F(1, 128)=110.73, p<0.001$], and time [$F(1, 128)=26.31, p<0.001$], with ethanol-exposed animals being less active than those given saline, adults exhibiting fewer crossovers between compartments than all 3 groups of adolescents, and animals tested 5 minutes after injection being generally less active than those tested at the 30 minutes after injection interval regardless

of their treatment (Fig. 1, bottom panels). However, in contrast to overall social activity, no significant interactions between age, treatment, and time were observed in terms of general locomotion indexed in this context. Similar age and time main effects were revealed in a 4 (age)×2 (time)×2 (sex) ANOVA conducted on overall number of crossovers in saline-injected animals only [$F(3, 64)=11.56, p<0.001$; and $F(1, 64)=12.69, p<0.001$, respectively].

Impairment of Locomotor Activity—Given these age and test interval effects evident even in saline-treated animals, ethanol-induced impairment of overall locomotor activity was expressed as the percent change in the number of crossovers relative to saline controls (as described under Methods) and analyzed. Locomotor impairment in the context of social interactions, in contrast to social impairment, did not differ significantly as a function of age and postinjection time interval, although P28 animals showed a trend for a reduction in impairment of overall locomotor activity between 5 and 30 minutes after injection (see Fig. 2, right).

Experiment 2. Ethanol Levels in Brain and Trunk Blood Samples of Adolescent and Adult Rats Taken 5 and 30 Minutes After Injection

The results of Experiment 1 demonstrated that all age groups showed equivalent ethanol-induced social impairment when tested at shorter injection-test interval (i.e., 5 minutes), suggesting that the initial magnitude of social inhibition to ethanol is comparable across age. Social inhibition was still pronounced at the 30-minute injection-test interval in adults, but was diminished in an age-related manner at younger ages. One possible explanation for these results is a faster emergence of acute tolerance at younger ages. Alternatively, it is possible that age-related changes in ethanol distribution or other pharmacokinetic factors could contribute to these findings. To address the latter possibility, Experiment 2 assessed ethanol concentrations in brain and trunk blood samples of P28, P35, P42, and P70 rats taken 5 or 30 minutes after administration of the 1 g/kg test dose of ethanol.

Methods—Brain and BECs were assessed in male and female animals at all 4 ages: P28, P35, P42, and P70. Each animal was injected with 1.0 g/kg ethanol, placed individually into a holding cage for 5 or 30 minutes and then decapitated. Trunk blood samples were collected using heparinized tubes. The brain was quickly removed, rinsed, and placed into aluminum foil. Blood and brain samples were then rapidly frozen and maintained at -80°C until analyses of blood and brain ethanol content. 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 minutes 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. Whole brain samples were thawed at time of analysis and 2 mL of cold distilled water added per gram of brain tissue before homogenizing the sample at 5°C . The brain samples were then analyzed for ethanol content using the same assay procedure as used for blood.

Brain ethanol concentrations and BECs were analyzed using separate 4 (age)×2 (postinjection time)×2 (sex) ANOVAs. Seven animals were placed into each of the 16 experimental groups in this experiment.

Results—Brain ethanol concentrations and BECs did not differ as a function of age or sex, with ethanol levels in both brain [$F(1, 96)=86.43, p<0.001$] and trunk blood [$F(1, 96)=112.01$,

$p < 0.001$] being significantly higher at 5 than 30-minute postinjection times. As seen in Fig. 3, BrECs were comparable with BECs at both postinjection time points.

Experiment 3. Acute Tolerance to Ethanol-Induced Social Impairment in Adolescent and Adult Rats

Although BrECs and BECs were significantly higher at 5 than at 30 minutes after administration of the test ethanol dose of 1 g/kg (Experiment 2), no age-related differences in ethanol levels in blood and brain emerged that could explain the rapid recovery from social impairment seen in P28 adolescents and the lack of such recovery in rats at P42 and P70 (Experiment 1). Together, these 2 sets of data add evidence to the hypothesis that the decline in ethanol-related social impairment from 5 to 30 minutes after administration in the younger animals (P28, P35) reflects a rapid emergence of acute tolerance at these ages. This hypothesis was further examined in Experiment 3 by evaluating expression of acute tolerance, as indexed by an increasing dissociation between ethanol levels in blood or brain and a more rapid decline in ethanol-induced impairment following a given dose of ethanol (Khanna et al., 2002; Radlow, 1994). Therefore, the relationship between BrECs and degree of social impairment was assessed in individual animals at P28, P35, P42, and P70 during the early recovery period following acute ethanol challenge.

Method—A total of 48 litters provided 240 male and female offsprings to serve as experimental subjects and 240 to serve as partners in Experiment 3. Subjects were injected either with 0 (saline) or 1 g/kg ethanol and were tested either immediately (0 minute) or 15 or 30 minutes following injection, with equal numbers of males and females placed into each treatment and age group (testing at P28, P35, P42, and P70). Rats in the 30-minute time interval group were injected with ethanol or saline and then placed individually into holding cages for 30 minutes before testing; subjects in the 15-minute group were placed into individual cages for 15 minutes, injected, and returned to individual holding cages for 15 more minutes, whereas animals tested immediately after saline or ethanol administration were placed into individual holding cages for 30 minutes and then injected and placed into the testing apparatus. In contrast to the 10-minute social interaction test in Experiment 1, animals encountered nonmanipulated partners for only 5 minutes in Experiment 3 to minimize time interval between the onset of behavioral testing and brain sample collection for later analysis of ethanol content. Overall social activity (sum of the frequencies of social investigation, contact behavior, and play) and locomotor activity (number of crossovers between the compartments) were scored. Immediately after the social interaction test, experimental subjects were killed by decapitation, and brains were collected and frozen for later BrEC analysis (see Experiment 2). Therefore, the design of Experiment 3 was a 4 (age) $\times$ 2 (treatment) $\times$ 3 (postinjection test interval) $\times$ 2 (sex) factorial, with 5 animals being placed in each of the 48 groups defined by this experimental design.

As in Experiment 1, overall social activity (sum of the frequencies of social investigation, contact behavior, and play) and locomotor activity (as indexed in this context by number of crossovers between the compartments) were scored and analyzed using separate 4 (age) $\times$ 2 (treatment) $\times$ 3 (time) $\times$ 2 (sex) ANOVAs. Following these analyses of raw data, acute tolerance was measured as an output function relating BrEC and associated impairment for each individual animal (see Radlow, 1994, for details). Brain ethanol concentrations at the different time points for each group were converted to percentages of the average maximum BrEC at each age (which was observed 5 minutes following ethanol administration under all circumstances), with this maximum BrEC expressed as 100%. Social and locomotor impairment, indexed as decline scores in overall social and locomotor activity relative to saline controls (see Experiment 1), were likewise converted to percentages of the maximum impairment (demonstrated by all experimental groups tested immediately after ethanol

administration), with this maximum impairment expressed as 100% within each experimental group (Khanna et al., 2002). Percent impairment values were subtracted from percent BrEC values for each individual animal at each time point [BrEC (% maximum)– impairment (% maximum)] and plotted as an output function over time separately for each age group. To assess acute tolerance, regression analyses were performed to determine the slopes of the regression of this output function at each age and whether slopes differed significantly from 0. Acute tolerance was defined as greater output values over time (i.e., ethanol-induced impairment declining more rapidly than BrECs) and was indexed as a positive slope (i.e., significantly greater than 0) of the linear regression that related these variables.

Results

Overall Social Activity—As seen in Fig. 4 (top panels), overall social activity differed as a function of age, treatment, and postinjection interval. A 2 (age)×2 (treatment)×3 (time)×2 (sex) ANOVA revealed significant main effects of age [$F(3, 192)=6.87, p<0.001$], treatment [$F(1, 192)=330.23, p<0.0001$], and time [$F(2, 192)=32.69, p<0.001$], as well as a significant age×treatment×time interaction [$F(6, 192)=2.35, p<0.05$]. Overall social activity of saline controls did not differ as a function of age or time in Experiment 3, whereas ethanol-treated animals demonstrated pronounced age-related and time-dependent differences in overall levels of social activity. Ethanol similarly decreased social activity of P28, P35, P42, and P70 rats tested immediately following administration, with only P28 animals recovering to show no social impairment at 30 minutes after injection—findings reminiscent of those presented in Experiment 1.

Locomotor Activity—General locomotor activity in the context of social interactions differed as a function of age [$F(3, 192)=6.88, p<0.001$], treatment [$F(1, 192)=138.18, p<0.0001$], and time [$F(2, 192)=13.92, p<0.0001$] (see Fig. 4), with no significant interactions involving these variables. In general, P28 animals demonstrated more locomotor activity in this social context than P42 and P70 rats, with ethanol-treated animals showing less locomotor activity than saline-treated controls, and animals tested immediately or 15 minutes after ethanol or saline administration demonstrating lower levels of locomotion than their counterparts tested 30 minutes after administration.

Acute Tolerance—When ethanol-induced impairment of social interactions was indexed as percentage of maximum social impairment (i.e., social impairment observed 5 minutes after injection) using the approach of Radlow (1994), these ethanol consequences declined more rapidly than BrECs (also indexed as percentage of maximum BrECs seen 5 minutes after injection) in P28 and P35 rats, suggesting acute tolerance development to the socially suppressing effects of ethanol. As shown in Fig. 5 (top panels), a similar dissociation between the behavioral and BrEC data did not emerge in late adolescents (P42) and adults (P70), with animals of these ages demonstrating similar declines in social impairment and BrECs. Regression analyses confirmed that P28 and P35 rats showed evidence for the expression of acute tolerance, as indexed by positive slopes of the linear regression of the output function [BrEC (% maximum)– impairment (% maximum)] over time, with these slopes differing significantly from 0 (see Table 1). Acute tolerance to ethanol-induced social impairment did not emerge in late adolescents and adults, with the slopes of the linear regression not differing significantly from 0 at either age (Table 1). Given that no sex-related differences in the development of acute tolerance were observed, the data are collapsed across this variable.

No dissociations between BrECs and locomotor impairment in the social context were observed at any of the ages (see Fig. 4, bottom panels), nor was there evidence of acute tolerance to the suppressing effects of ethanol on total number of crossovers when using the slope of the linear

regression of the output function to index acute tolerance, with these slopes not differing from 0 in any age group (see Table 1).

DISCUSSION

To our knowledge, this is the first study to demonstrate greater expression of acute tolerance in adolescent than adult animals at moderate ethanol exposure levels within the BEC range currently accepted to define human binge drinking (i.e., a pattern of drinking that brings BEC to 80 mg/dL or above, NIAAA, 2004). In the present study, young adolescent rats, in contrast to their older adolescent counterparts and adults, developed compensatory adaptations to the impairment of social behavior by ethanol within 30 minutes, a time interval over which their BrECs or BECs declined from about 120 to 80 mg%. This expression of acute tolerance among early adolescents extends previous ontogenetic findings obtained using high, hypnotic doses of ethanol (Draski et al., 2001; Silveri and Spear, 1998) to adaptations occurring in response to a moderate dose of ethanol (1 g/kg i.p.) and producing BECs of relevance to exposure levels emerging with binge use among human adolescents.

The results of Experiment 1 demonstrated marked age-dependent and time-dependent effects of ethanol on overall social activity. Testing younger adolescents (P28 and P35) at 30 minutes following administration of 1 g/kg revealed little evidence of the social inhibition that was apparent in more mature animals (P42 and P70). These results are in agreement with previously described age-related increases in sensitivity to the suppressing effects of ethanol on social behavior from adolescence to adulthood (Varlinskaya and Spear, 2002). However, all age groups showed equivalent ethanol-induced social impairment when tested at a shorter injection-test interval (i.e., 5 minutes), suggesting that initial sensitivity to ethanol-induced social inhibition is more comparable across age.

One could argue that the suppressant effects of ethanol on social behavior may have been associated with a general sedative effect of ethanol, given that the total number of crossovers between compartments (used as an index of overall locomotor activity in this study) was also decreased after ethanol administration. However, there was little evidence of age-dependent or time-dependent differences in the ethanol-induced decrease of total crossovers at the 1 g/kg test dose in Experiment 1, and, in contrast to social impairment, suppression of crossovers did not differ among age groups 30 minutes after ethanol administration. Furthermore, with this measure, even the youngest adolescent group showed only a non-significant tendency for less locomotor impairment at the 30-minute injection-test interval, whereas they demonstrated total recovery of social activity at this time point. Likewise, P35 adolescents showed a significant reduction of social but not locomotor impairment over time. Therefore, it seems unlikely that ethanol-induced changes in social behavior are simply related to overall sedation.

Ontogenetic differences in sensitivity to the suppressing effects of ethanol on social behavior in Experiment 1 may be related, at least in part, to the decrease in brain water content across ontogeny (Vernadakis and Woodbury, 1962). Ethanol concentrations in the brains of younger animals potentially could be lower than in the brains of their older counterparts simply as a function of this developmental decline in brain water content. This presumed dilution of ethanol by higher brain water content during early and mid adolescence would be expected to diminish the functional impact of a given ethanol dose on a behavioral measure. However, the results of Experiment 2 do not support this possibility, in that similar time-related decreases in brain and blood ethanol levels were evident at all ages. In these assessments, BrECs did not differ from BECs either 5 or 30 minutes after injection at any of the test ages. These results are in agreement with other reports that ethanol levels in trunk blood or retro-orbital sinus blood accurately reflect ethanol concentrations in brain as early as several minutes after administration in mice (Gehle and Erwin, 2000; Ponomarev and Crabbe, 2002) and rats

(Sunahara et al., 1978). In some studies, ontogenetic differences in ethanol distribution and metabolism have been reported. Ontogenetic increases in ethanol metabolism have frequently been reported in studies conducted across the early postnatal period (e.g., Kelly et al., 1987; Silveri and Spear, 2000), although by adolescence the rates of ethanol metabolism have been found to reach (Silveri and Spear, 2000) or slightly exceed (Brasser and Spear, 2002) those of adult animals. Some differences in blood or brain ethanol levels between adolescent and adult rats have been reported following administration of relatively high doses of ethanol (Little et al., 1996; Silveri and Spear, 2000), with no such age-related differences in BrECs or BECs emerging, however, at low doses of ethanol, including the 1 g/kg dose used in the present study (Kelly et al., 1987; Silveri and Spear, 2000; Varlinskaya and Spear, 2002).

Given the lack of age-related differences in BECs or BrECs at the 30-minute postinjection interval, the most parsimonious explanation of the pronounced decline in ethanol-related social impairment from 5 to 30 minutes after administration in P28 and P35 animals is a rapid emergence of acute tolerance at these ages, with social impairments declining more rapidly than BrECs. This possibility was directly tested in Experiment 3 using the relationship between BrEC and ethanol-induced social impairment as a measure of acute tolerance (Khanna et al., 2002; Radlow, 1994). In this study, an ontogenetic decline in emergence of acute tolerance to social inhibition (but not to the ethanol-induced suppression of locomotor activity) was observed. Only early (P28) and mid (P35) adolescent rats showed an increasing time-dependent dissociation between BrECs and ethanol-induced social impairment following the relatively moderate dose of 1 g/kg ethanol, with social impairment declining faster than BrECs. Older adolescents and adults did not show acute tolerance to ethanol-induced social inhibition, with no acute functional tolerance emerging to the suppressing effects of ethanol on locomotor activity in this test at any age. Therefore, considerable ontogenetic differences in the development of acute tolerance to ethanol-induced social inhibition are evident even within the adolescent period, with only early and mid but not late adolescents showing rapid emergence of this ethanol-related compensatory response. This finding of an ontogenetic decline in acute tolerance across the adolescent period is reminiscent of previous ontogenetic studies (Draski et al., 2001; Silveri and Spear, 1998). However, in the present experiments acute tolerance to ethanol-induced social inhibition emerged rapidly (within a 30-minute period after ethanol administration) and was observed using a relatively modest dose of 1 g/kg, whereas previous ontogenetic declines in acute tolerance to the sedative effects of ethanol were examined at substantially higher doses (2.5–5.0 g/kg) and over a longer period of time (up to 7 hours).

The development of acute tolerance to ethanol-induced social inhibition but not to the impairment in locomotor activity expressed in this context was not surprising, given a number of experimental findings demonstrating that acute tolerance is task dependent and develops at different rates to different effects of ethanol even in the same animal. For instance, lines of mice selectively bred for high and low acute functional tolerance to ethanol-induced balance on a dowel (HAFT and LAFT, respectively), did not differ in the acute tolerance they developed to a number of other ethanol effects, including the loss of the righting reflex, hypothermia (Erwin et al., 2000), and motor in coordination on a rotorod (Deitrich et al., 2000). As another example, acute tolerance to the loss of the righting reflex, but not to ethanol-induced disruption of motor coordination, was observed in high, low, and control alcohol-sensitive rats selected on the basis of their sensitivity to ethanol-induced hypnosis (Draski et al., 2001). Similarly, Silveri and Spear (2001) reported the development of acute tolerance to ethanol-induced motor impairment, but not to ethanol-related hypothermia in Sprague–Dawley rats. Taken together, these findings demonstrate that acute tolerance to ethanol is response-specific and test method-specific and support the suggestion that neural adaptations to different ethanol effects may be separable (Erwin et al., 2000). Indeed, it should be recognized that, although no acute tolerance to ethanol-induced suppression of locomotor activity emerged

within a social context, acute tolerance to ethanol-associated locomotor impairment could be expressed in other, nonsocial contexts, including situations more typically used to assess locomotor activity.

Although consistent with previous ontogenetic studies (Draski et al., 2001; Silveri and Spear, 1998), the lack of acute tolerance expression in late adolescents and adults in the present study was rather surprising, given that acute tolerance has been observed in adult rats to a number of ethanol effects, including ethanol-induced motor impairment (Khanna et al., 2002; LeBlanc et al., 1975; Lumeng et al., 1982; Silveri and Spear, 2001) and sedation (Silveri and Spear, 2004). A number of factors could contribute to the differential expression of acute tolerance in adults across studies. For instance, experimental subjects in the present study and others where adults did not express acute tolerance (e.g., Draski et al., 2001; Silveri and Spear, 1998) were group-housed rats not manipulated in any way before the acute tolerance test. In the studies that demonstrated the emergence of acute tolerance in adults, rats were either individually housed or trained to perform a target task, with these experimental procedures often requiring substantial training and/or application of stressors. For instance, LeBlanc et al. (1975) trained socially isolated rats for several weeks to remain on a moving belt, with animals being shocked for placing a paw on a grid, whereas in the Lumeng et al. (1982) study individually housed animals required 10 days of training on a jumping apparatus to avoid or escape foot shock. Similarly, Silveri and Spear (2001), although using group-housed animals, trained them for 5 days in a swim escape task before acute tolerance assessment. Therefore, it is possible that under circumstances involving pretest training and/or manipulations, as well as isolate housing, the emergence of acute tolerance may be fostered, whereas test circumstances involving group-housed animals who are nonmanipulated before testing may not favor expression of acute tolerance in adulthood. Indeed, relatively subtle manipulations have been reported to influence expression of acute tolerance among adult animals, with, for instance, an i.p. injection of saline administered to adult rats the day before testing being sufficient to induce expression of acute tolerance to the sedative effects of ethanol that was not otherwise expressed with these testing methods in adults (Silveri and Spear, 2004).

The greater expression of acute tolerance in young animals, reflecting their enhanced ability to respond to ethanol challenge with compensatory adaptations, may stem from ontogenetic alterations in neural systems that continue to be elaborated during adolescence. Effects of ethanol are mediated through a number of neural systems, including enhancement of γ -aminobutyric acid (GABA) inhibitory effects through activation of GABA_A receptors and suppression of glutamatergic excitation through suppression of *N*-methyl-D-aspartate (NMDA) receptor function (see Eckardt et al., 1998, for review). Attenuated sensitivity of adolescent animals to a number of acute ethanol effects, including ethanol-related anxiolysis (Varlinskaya and Spear, 2002), social inhibition (Varlinskaya and Spear, 2002, 2004), motor impairment (Hollstedt et al., 1980; Silveri and Spear, 2001; White et al., 2002), and sedation (Draski et al., 2001; Little et al., 1996; Moy et al., 1998; Silveri and Spear, 1998) may stem from age-related alterations in brain GABA and NMDA systems. Brain concentrations of GABA are low early in ontogeny and reach adult levels only in late adolescence (Vernadakis and Woodbury, 1962). The subtype composition (and, most probably, the properties) of GABA_A receptors in the immature brain also differs from that of adult brain (Fritschy et al., 1994; Laurie et al., 1992). In contrast to brain GABA systems, levels of glutamate and binding to the NMDA subtype of glutamate receptors peak in rats during the third postnatal week and decline gradually thereafter (Pruss, 1993), suggesting enhanced functional activity of certain brain glutamate systems in adolescents relative to adults.

Enhanced functional activity of NMDA receptor systems in immature animals may contribute not only to the decreased sensitivity of adolescent animals to a number of ethanol effects but to the enhanced expression of acute tolerance during adolescence. Indeed, acute tolerance to

ethanol-induced hypnosis was disrupted in adolescent animals by the NMDA antagonist (+)MK-801 but not by GABA agonist muscimol (Silveri and Spear, 2004), although this disruption of acute tolerance expression was neither sufficient nor necessary to eliminate ontogenetic differences in ethanol sensitivity, at least in terms of ethanol-induced sedation. These data, suggesting a role for NMDA receptors in the development of acute tolerance in adolescents (Silveri and Spear, 2004), are reminiscent of findings of Khanna et al. (2002), who reported that pretreatment with (+)MK-801 blocked the development of acute tolerance to ethanol-induced motor impairment in adult rats.

A greater propensity of early adolescents to develop acute tolerance may contribute to their relative resistance to many effects of ethanol that serve as cues to limit drinking. This attenuated sensitivity to ethanol may serve as a permissive factor to allow relatively high levels of alcohol consumption, placing young adolescents at risk for extensive alcohol use. And indeed, levels of ethanol use are often high during adolescence, with adolescents consuming on average 4 to 5 drinks per occasion (Substance Use and Mental Health Services Administration, 2003) and 14% of eighth graders, 26% of tenth graders, and almost one-third of twelfth graders (30%) reporting binge drinking during the past 2 weeks (Johnston et al., 2001). By focusing on ontogenetic changes in ethanol adaptations occurring at BECs within the range of human binge drinking, the findings of the present study may have important implications for our understanding of the drinking patterns of human adolescents and the factors that encourage the frequent emergence of binge-like patterns of drinking in this age group.

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Fig 1. Overall social activity (top) and locomotor activity (bottom) for early [postnatal day (P)28], mid (P35), and late (P42) adolescent and adult (P70) rats tested either 5 or 30 minutes following administration of 0 (saline) or 1 g/kg ethanol (EtOH) in Experiment 1. *Significant difference from corresponding saline controls, with $p < 0.05$.

Fig 2. Social impairment (left) and impairment of locomotor activity (right) calculated as the percentage decline in overall social activity or in overall number of crossovers, respectively, after the 1 g/kg ethanol test dose relative to the corresponding saline control group for early postnatal day [(P)28], mid (P35), late (P42) adolescent and adult (P70) rats tested 5 or 30 minutes following ethanol administration in Experiment 1. *Significant change in social impairment from minutes 5 to 30, with $p < 0.05$.

Fig 3.
Brain and blood ethanol concentrations for early [postnatal day (P)28], mid (P35), and late (P42) adolescent and adult (P70) rats 5 or 30 minutes following administration of the challenge dose of 1 g/kg ethanol in Experiment 2.

Fig 4. Overall social activity (top) and locomotor activity (bottom) for early [postnatal day (P)28], mid (P35), and late (P42) adolescent and adult (P70) rats tested either immediately (0 minute) or 15 or 30 minutes following administration of 0 (saline) or 1 g/kg ethanol (EtOH) in Experiment 3. *Significant difference from corresponding saline controls, with $p < 0.05$.

Fig 5.

Changes in ethanol induced impairment and brain ethanol concentrations (top) and impairment of locomotor activity and brain ethanol concentrations (BrECs) over time in early [postnatal day (P)28], mid (P35), and late (P42) adolescent and adult (P70) rats in Experiment 3. Social impairment and impairment of locomotor activity are indexed as percentage of maximum social impairment or impairment of locomotor activity, correspondingly. Brain ethanol concentrations are converted to percentage of maximum BrEC.

Table 1
Acute Tolerance to Ethanol-Induced Impairment Measured as an Output Function Relating BrECs and Impairment in Adolescent and Adult Rats

Age	Social impairment		Motor impairment	
	Slope	<i>F</i> (1, 28)	Slope	<i>p</i>
P28	1.99+0.54	13.28	0.10+0.9	0.01
P35	1.16+0.30	14.57	0.44+0.57	0.59
P42	0.08+0.54	0.02	0.13+0.73	0.03
P70	0.16+0.43	0.13	0.20+0.61	0.11

Slopes significantly different from 0 are presented in bold.