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Comparison of ethanol locomotor sensitization in adolescent and adult DBA/2J mice

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Abstract

Rationale—The mammalian adolescent period is characterized by enhanced vulnerability to drug-induced neuroadaptations. Epidemiological evidence indicates that individuals who start drinking alcohol during adolescence are four times more likely to develop alcohol dependence in adulthood, but little is known about the adaptive mechanism(s) that may underlie this observation. Behavioral sensitization in rodents is a model of neurobehavioral plasticity that occurs following repeated drug exposure and may underlie components of addiction.

Objectives—The goal of this study was to determine if adolescent mice are differentially sensitive to ethanol-induced locomotor sensitization as compared to adults.

Materials and methods—Adolescent and adult DBA/2J mice were treated with saline or ethanol (1.0, 1.5, 2.0, 2.5 g/kg) for 7, 11, or 15 days and tested for acute and sensitized locomotor activity. Blood ethanol clearance (BEC) was also assessed 10, 60, and 180 min following treatment with ethanol 2 g/kg.

Results—Adolescent mice were more sensitive than adult mice to the acute locomotor activating effects of ethanol. However, adolescent mice were less sensitive than adult mice to locomotor sensitization, as only the highest dose of ethanol (2.5 g/kg) induced sensitization in the adolescent mice, while lower doses of ethanol elicited sensitization in the adult mice. The differential response to ethanol sensitization was not related to duration of treatment or differential BEC.

Conclusions—These results indicate that adolescent mice are less sensitive to ethanol sensitization, and this blunted behavioral response in adolescents might reflect differential ethanol-induced neurobehavioral adaptations.

Keywords

Alcohol; Sensitization; Ethanol; Adolescent; Adult; Neuroadaptation; Locomotor

Introduction

Adolescence is a critical period of development during which children and young animals undergo adaptive changes in behavior and neurobiological systems that bring about the transition into adulthood. Behavioral changes include spending an increased amount of time engaged in social interaction with peers, taking part in risky behaviors, and exploring novel situations, while neurobiological changes include remodeling in the cortex and mesolimbic

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regions such that glutamatergic and GABAergic neurotransmission is reduced and dopaminergic neurotransmission is increased (Spear 2000).

The behavioral and neurobiological adaptations that take place during this developmental stage cause the adolescent to be particularly vulnerable to experimenting with drugs of abuse and to subsequent drug-induced neuroadaptations (Crews et al. 2007; Spear 2002). The study of ethanol exposure during the adolescent period is important because it is known that people who start drinking during adolescence are four times more likely to become alcohol dependent as adults (Grant 1998). However, the mechanism (s) underlying this finding remain to be fully characterized.

Studies in rodents have shown that adolescents and adults are differentially sensitive to the effects of acute and chronic ethanol. Adolescent rodents are more sensitive to the effects of acute and chronic ethanol on measures of locomotor stimulation, anxiety, ataxia, spatial memory, conditioned place preference, and social interaction as compared to adult rats (Hefner and Holmes 2007; Markwiese et al. 1998; Philpot et al. 2003; Rajendran and Spear 2004; Varlinskaya and Spear 2002; Yttri et al. 2004). By contrast, other studies have shown that adolescent rodents are less sensitive than adult rats to the sedative and motor-impairing effects of ethanol, to ethanol-withdrawal-induced anxiety, and analgesia (Doremus et al. 2003; Hefner and Holmes 2007; Silveri and Spear 1998; Varlinskaya and Spear 2002; White et al. 2002). These differences in the sensitivity of adolescents to ethanol are important because it has been shown that a decreased response to acute alcohol challenge during adolescence is a potent predictor of future alcoholism (Schuckit 1993, 1994).

One model of neurobehavioral adaptations that occur following chronic ethanol exposure is locomotor sensitization. Sensitization is typically defined as a progressive increase in locomotor activity following repeated administration of a drug of abuse (Kalivas and Stewart 1991). The process of sensitization is thought to produce enduring adaptive changes in brain and behavioral function that may underlie components of addiction (Kalivas et al. 1998; Robinson and Berridge 2000). Research has shown that sensitization is mediated by an interconnected network of mesocorticolimbic brain regions (i.e., ventral tegmental area, nucleus accumbens, prefrontal cortex, amygdala, and thalamus) and neurotransmitter systems [i.e., dopamine, glutamate, and γ-aminobutyric acid (GABA)] (Kalivas 1995; Vezina and Kim 1999). These brain regions and neurotransmitter systems all undergo alterations during the adolescent developmental period (Kalivas 1995; Spear 2000; Vezina and Kim 1999). Thus, sensitization models are useful tools to determine if adolescent vulnerability to addiction involves differential sensitivity to neurobehavioral changes that occur with repeated drug use.

Various protocols have been used to induce ethanol locomotor sensitization, all of which involve repeated administration of ethanol over a number of days. The dose of ethanol used to induce locomotor sensitization commonly ranges from 1.5 to 2.5 g/kg, administered for 4 to 21 days (Broadbent and Harless 1999; Broadbent and Weitemier 1999; Fish etal. 2002; Itzhak and Martin 2000; Lessov etal. 2001; Meyer and Phillips 2003; Miquel et al. 2003; Quadros et al. 2003). In adult DBA/2J mice, sensitization develops after three ethanol exposures and persists up to 68 days after the final ethanol treatment (Fish et al. 2002; Lessov et al. 2001). These studies indicate that long-lasting neurobiological changes occur during sensitization. Importantly, ethanol locomotor sensitization has not been studied in adolescent rodents.

The present study was designed to examine potential developmental differences in sensitivity to the neurobehavioral adaptations that occur during the induction of ethanol sensitization. Given the differential behavioral responses to ethanol in adolescents and adults, this study sought to fully characterize ethanol dose response and time course for sensitization in both adolescent and adult mice.

Materials and methods

Animals

Male 3-week old (adolescent) and 8-week old (adult) DBA/2J mice (Jackson Laboratories, Bar Harbor, ME, USA) were housed in groups (four animals per cage) in standard Plexiglas cages with food (Purina Rodent Chow) and water available ad libitum. The colony was maintained at 27°C on a 12-h light/dark cycle, with the lights on at 10 P.M. The behavioral experiments were conducted during the dark portion of the cycle. Mice were handled and weighed daily for 1 week before and for the duration of the experiment. Animals were under continuous care and monitoring by the Division of Laboratory Animal Medicine at University of North Carolina-Chapel Hill, and all procedures were carried out in accordance with the *NIH Guide to Care and Use of Laboratory Animals* (National Research Council 1996) and institutional guidelines.

Behavioral apparatus

The locomotor activity (horizontal distance traveled, in centimeters) of adolescent and adult mice was measured in eight covered Plexiglas chambers (30 cm^2, Med Associates, Georgia, VT, USA). Two sets of 16 pulse-modulated infrared photobeams were located on opposite walls to record ambulatory movements in the *x*-*y* (horizontal) plane. All software settings were the same for adults and adolescent mice. The activity chambers were computer-interfaced (Med Associates) for data sampling at 100-ms resolution.

Behavioral procedures

Mice were adapted to the colony and to handling for 1 week (adolescents=P28; adults=P63). On locomotor testing days, mice were taken in the home cage to the testing room at least 30 min before the session to habituate to the testing room. The first 2 days of the each experiment were habituation days (H1 and H2). On these days, all mice received an intraperitoneal (IP) injection of saline and were immediately placed in the locomotor chamber for the 10-min session.

Experiment 1a: acute locomotor activity—Adolescent and adult mice received an IP injection of 0, 1.0, 1.5, 2.0, or 2.5 g/kg ethanol (*n*=8 per age group per ethanol dose) and were immediately placed in the locomotor chamber for the 10-min session.

Experiment 1b: sensitization dose response—Following the acute locomotor session on day 1 (D1), mice receivedthe assigned ethanol dose (0, 1.5, 2.0, 2.5, or 3.0 g/kg IP) once daily for 9 days (D2-D10) in the home cage. On day 11 (D11), the mice were tested for locomotor sensitization. Mice were injected with 0, 1.0, 1.5, 2.0, or 2.5 g/kg ethanol (IP) and placed in the locomotor chamber for 10 min (Lessov and Phillips 1998).

Experiment 2a: sensitization time course—On day 1 (D1), the mice received an IP injection of 0, 2.0, or 2.5 g/kg ethanol (*n*=8 per age group per ethanol dose per length of treatment) and were immediately placed in the locomotor chamber for 10 min. For the following days (D2-D6 or D2-D10), mice received the assigned ethanol dose (0, 2.5, or 3.0 g/kg IP) once daily and were returned to the home cage. On day 7 or 11 (D7 or D11), the mice were tested for locomotor sensitization. Mice were injected with 2.0 or 2.5 g/kg ethanol (IP) and placed in the locomotor chamber for 10 min.

Experiment 2b—Mice received ethanol 2.0 g/kg on D1, followed by daily (D2-14) treatment with ethanol 2.5 g/kg (*n*=8 per age group). On day 15, mice were tested for locomotor sensitization to ethanol 2.0 g/kg.

Experiment 3: blood ethanol determination—Tail blood was collected from adolescent and adult mice at 10, 60, and 180 min after an initial ethanol (2.0 g/kg) injection (D1; *n*= 6-8 per age group). Mice were then treated with ethanol (2.5 g/kg) for the following nine days (D2-D10). Tail blood was collected again on day 11 (D11) of ethanol (2.0 g/kg; *n*=6-8 per age group) administration at 10, 60, and 180 min post-injection. Individual blood samples were centrifuged, and 5 μl of plasma from each sample was analyzed to determine blood ethanol concentration using an AM1 Alcohol Analyzer (Analox Instruments, Lunenburg, MA, USA).

Drugs

Ethanol (95% *w*/*v*) was diluted in saline (0.9%) to a concentration of 20% (*v*/*v*) and injected at different volumes to achieve the appropriate dosage (i.e., 2.0 and 2.5 g/kg). Control animals received 0.9% saline.

Behavioral measures and data analysis

Horizontal distance traveled (in centimeters) during the 10-min session was calculated from the number of photobeam breaks and presented as mean± SEM. The distance traveled on habituation days 1 and 2 was compared between adolescent and adult mice using an unpaired *t* test. Statistical significance was defined as $p \leq 0.05$ in all experiments.

Experiment 1a: acute locomotor activity—The total distance traveled (in centimeters) after an acute injection of saline or ethanol was examined using two-way analysis of variance (ANOVA) with age (adolescent and adult) and ethanol dose as factors. Post hoc Tukey tests were used to determine between-group differences.

Experiment 1b: Sensitization dose response—Distance traveled (in centimeters) was analyzed within the adolescents and adults using three-way repeated measure (RM) ANOVA, with age (adolescent and adult), day (D1 and D11), and ethanol dose as factors. Significant interactions were followed with analysis by lower order (e.g., two-way) ANOVA where appropriate. Sensitization was defined as activity on day 11 being significantly greater than activity on day 1 within an ethanol dose, as determined by post hoc Tukey tests. This within-group definition of sensitization was applied because it was observed that groups of adolescent and adult mice treated repeatedly with saline and given acute ethanol (1.0, 1.5, 2.0, or 2.5 g/kg) on day 11 displayed an equivalent locomotor response to the mice treated with acute ethanol on day 1 (data not shown). The data were presented as mean (±SEM).

To determine if the magnitude of sensitization to ethanol 2.5 g/kg differed in the adolescents and adults, the locomotor activity from day 11 was expressed as a percent increase from day 1 activity. An unpaired *t* test was used to compare the magnitude of sensitization between the age groups.

To determine if degree of sensitization was influenced by acute response to ethanol, a linear regression analysis was conducted comparing locomotor response to acute ethanol (D1) versus the sensitization test day (D11) for two doses of ethanol (2.0 and 2.5 g/kg) within each age group.

Experiment 2a: sensitization time course—Groups of mice were evaluated for distance traveled (in centimeters) following treatment with ethanol (2.0 or 2.5 g/kg) using four-way RM ANOVA, with age (adolescent and adult), ethanol dose, treatment duration (7 or 11 days) and test day (acute and sensitization) as factors. Significant interactions were followed with analysis by lower order (e.g., two-way) ANOVA where appropriate. Sensitization was defined as activity on day 7 or 11 (D7 or D11) being significantly greater than activity on day 1 (D1).

Experiment 2b—The mice treated with ethanol 2.0 g/kg for 15 days were analyzed using RM two-way ANOVA, with age (adolescent and adult) and treatment day (D1 or D15) as factors. Sensitization was defined as activity on day 15 being significantly greater than activity on day 1 within an age group, as determined by post hoc Tukey tests. The data were presented as mean (±SEM).

Experiment 3: blood ethanol clearance—The blood ethanol clearance (BEC) data were analyzed using 3-way RM ANOVA, with age (adolescent and adult), day (D1 and D11), and time (10, 60, and 180 min) post-ethanol injection as factors. Significant interactions were followed with analysis by lower order (e.g., two-way) ANOVA to determine whether the BEC following acute ethanol and following chronic ethanol treatment was responsible for age-dependent differences in sensitization. Post hoc Tukey tests were used to extract group differences.

Results

Basal activity and response to acute ethanol

Since adolescent mice are differentially sensitive to acute effects of ethanol as compared to adults (Hefner and Holmes 2007), we first examined basal locomotor activity and response to acute ethanol (1.0-2.5 g/kg). On the habituation days, no differences in locomotor activity were observed between the adolescent and adult groups (p=0.29; adolescents, 3,011±234 cm; adults, 2,670±210 cm). Adolescent and adult mice showed equal saline-induced locomotor activity but different locomotor response to acute ethanol treatment (Fig. 1). Two-way ANOVA showed that adolescent mice were more sensitive to the acute locomotor activating effects of ethanol as compared to adults. There was a significant main effect of age [F(1, 61)=11.01, p=0.002], a significant main effect of ethanol dose [F(4, 61)=10.05, p<0.001], and a significant interaction [F(4, 61)=3.02, p= 0.024]. In the adolescents, ethanol doses of 1.5, 2.0, and 2.5 g/kg significantly increased locomotor activity (Fig. 1). Overall, these results indicate that adolescent DBA/2J mice are more sensitive than adult mice to the acute locomotor activating effects of ethanol in a dose-dependent manner.

Sensitization: dose response

Following acute ethanol treatment, adolescent and adult mice were tested for locomotor sensitization (ethanol 0-2.5 g/kg). Locomotor sensitization was defined as a significant increase in locomotor activity on day 11 compared to day 1 within each dose, as determined by post hoc Tukey tests. Three-way ANOVA of age × ethanol dose × day revealed significant main effects of the between-subject variables age [F(1, 56)=9.68; p=0.003] and ethanol dose [F(4, 56)=48.81; p<0.001]. A significant main effect was also noted for the within-subject factor day [F(1, 56)=148.43; p<0.001] along with a significant day × age interaction [F(1, 56)=4.31; p<0.05], a significant day × dose interaction [F(4, 56)=38.32; p<0.001], and a significant day × age × dose interaction [F(4, 56)=5.88; p=0.001]. Due to the three-way interaction, locomotor activity was analyzed separately for adolescent and adult mice to examine age-dependent sensitization. Overall, adolescent mice appeared to be less sensitive to ethanol sensitization as shown by lack of response to doses of ethanol that induced sensitization in adult mice (1.5 and 2.0 g/kg; Fig. 2). Within the adolescents, there was a significant main effect of dose [F(4, 29) =25.00, p<0.001], a significant main effect of treatment day [F(1, 29)=38.59, p<0.001], and a significant interaction [F(4, 29)=19.91, p<0.001]. Sensitization was only observed at the 2.5 g/kg ethanol dose (Fig. 2a; p<0.001). In the adults, there was also a significant main effect of dose [F(4, 28)=21.98, p<0.001], a significant main effect of treatment day [F(1, 28)=129.89, p<0.001], and a significant interaction [F(4, 28)=17.88, p<0.001]. The adults showed sensitization at ethanol doses of 1.5, 2.0, and 2.5 g/kg (Fig. 2b; p <0.001). These results indicate

that the adolescent mice are less sensitive than the adult mice to ethanol sensitization, as they require a higher dose of ethanol (2.5 g/kg) to exhibit locomotor sensitization.

To determine if the magnitude of sensitization to ethanol 2.5 g/kg was greater in the adolescents than in the adults, the locomotor activity from day 11 was expressed as a percentage of the locomotor activity from day 1 (data not shown). Comparison of the adolescent and adult level of sensitization did not differ (p=0.53; adolescents, 438.9±118% increase; adults, 357.3±48% increase). These data indicate that the adolescent and adult mice display sensitization to ethanol 2.5 g/kg to the same degree.

Sensitization: time course

To further assess age-dependent differences, we next examined the effect of different ethanol treatment durations on the induction of locomotor sensitization to ethanol (Fig. 3). Sensitization was defined as a significant increase in distance traveled on the final day of treatment (day 7 or 11) as compared to a single acute treatment (day 1). The time course of the induction of ethanol sensitization was evaluated after ethanol (2.0 and 2.5 g/kg) tests to compare response to doses that demonstrated differential age-dependent sensitivity (shown in Fig. 2).

Four-way ANOVA comparing age × dose × treatment duration × test day identified significant main effects for the between-subjects factors of age [$F(1,54)=5.84$; $p=0.02$] and ethanol dose [$F(1,54)=15.89$; $p<0.001$]. There was no main effect of treatment duration and no interactions between age, dose, and duration. A significant main effect was also identified for the within-subject factor test day [$F(1,54)=181$; $p=0.001$]. Analysis of the two-way interactions showed that the main effect of test day (i.e., acute vs sensitization test) was dependent on age [$F(1, 54)=9.44$; $p=0.003$] and dose [$F(1, 54)=59.44$; $p<0.001$]. Three-way interaction terms showed that the effect of test day (i.e., acute vs sensitization test) was dependent on the level of age and dose [$F(1, 54)=12.9$; $p=0.001$] as well as treatment duration and dose [$F(1, 54)=4.07$; $p<0.05$]. Based on these significant interactions, the sensitization data were analyzed separately for each dose and duration.

For mice treated with ethanol 2.0 g/kg for 7 days, two-way RM ANOVA revealed a significant main effect of test day [$F(1, 31)=12.44$; $p=0.003$] and a significant test day × age interaction [$F(1, 31)=6.75$; $p=0.02$]. Multiple comparisons showed that, overall, activity on day 7 was higher than activity on day 1 ($p=0.004$) and that this increase was dependent on an increase in locomotor activity in the adults on day 7 as compared to day 1 ($p<0.001$). These data show that the adults show locomotor sensitization to ethanol 2.0 g/kg after 7 days of treatment, while the adolescents do not show sensitization at this time point (Fig. 3a). For the 11-day time course of ethanol 2.0 g/kg, two-way RM ANOVA showed a significant main effect of test day [$F(1, 30)=8.32$; $p<0.02$] and a significant test day × age interaction [$F(1, 30)=26.46$; $p<0.001$]. Post hoc comparisons showed that, overall, locomotor activity on day 11 was significantly greater than activity on day 1 ($p<0.02$), and this effect was caused by a significant increase in the adult group on day 11 compared to day 1 ($p<0.001$; Fig. 3a). On day 1, the adolescents showed significantly more locomotor activity than the adults ($p<0.001$), while on day 11, the adults were more active than the adolescents ($p<0.02$). These results indicate that the adult group displayed sensitization to ethanol 2.0 g/kg following 11 days of treatment, while the adolescent group did not show sensitization. Furthermore, the adolescents displayed the expected greater acute locomotor activation to ethanol 2.0 g/kg, while the adults responded greater on the sensitization test day than the adolescents.

For ethanol 2.5 g/kg after 7 days of treatment, two-way ANOVA showed only a significant main effect of day [$F(1, 31)=78.15$; $p<0.001$], indicating that all mice displayed significantly increased activity on day 7 compared to day 1 ($p<0.001$). Similarly, ANOVA of treatment for 11 days with ethanol 2.5 g/kg showed a significant main effect of day [$F(1, 30)=108.85$;

$p<0.001$], indicating that all mice displayed sensitization (Fig. 3b). Taken together, these data show that regardless of duration of treatment, adolescent mice do not exhibit locomotor sensitization to ethanol 2.0 g/kg, while adult mice do exhibit sensitization to this dose of ethanol. Both adolescent and adult mice display locomotor sensitization to ethanol 2.5 g/kg.

The time course was extended to 15 days for the ethanol 2.0 g/kg to investigate whether treatment for a longer period of time would elicit sensitization in the adolescent group. Two-way ANOVA revealed a significant main effect of age [$F(1, 12)=11.94$; $p=0.005$], a significant main effect of test day [$F(1, 27)=22.38$; $p<0.001$], and a significant interaction [$F(1, 27)=7.94$; $p<0.02$]. Post hoc Tukey tests showed no difference between day 15 and day 1 within the adolescents ($p=0.23$), while there was a significant increase in activity on day 15 in the adults ($p<0.001$). These results indicate that the adolescent mice did not demonstration locomotor sensitization following 15 days of treatment with ethanol 2.0 g/kg, while the adult group did show sensitization. On the acute test day 1, the adolescents were significantly more active than the adults ($p<0.001$), while the age groups were not different on day 15 ($p=0.86$; data not shown).

Overall, the time-course experiment shows that adolescent mice do not display sensitization to ethanol 2.0 g/kg with up to 15 days of ethanol exposure, while the adult mice show sensitization following only 7 days of exposure. Both adolescent and adult mice exhibit sensitization to ethanol 2.5 g/kg with only 7 days of exposure. These results indicate that age-dependent ethanol sensitization is not effected by the duration of treatment but is mediated by the dose of ethanol.

Correlation: acute × sensitized locomotor response

To examine the possibility that the acute locomotor response to ethanol was predictive of the degree of locomotor activation after repeated treatment, a linear regression comparing day 1 (acute) activation and day 11 (sensitized) activation was performed in the adolescents and adults treated with either ethanol 2.0 or 2.5 g/kg. There was no significant correlation between acute locomotor activation and sensitized locomotor activation in the adolescents or the adults at either ethanol dose ($p>0.1$; data not shown). These data indicate that the acute locomotor response to ethanol 2.0 or 2.5 g/kg does not affect the magnitude of the sensitized locomotor response to repeated ethanol treatment in adolescent or adult mice.

Blood ethanol concentration

To examine whether differential age-dependent sensitization might be mediated by differences in ethanol clearance, an analysis of blood ethanol concentration (mg/dl) was conducted for sensitization treatment with ethanol 2.0 g/kg. Importantly, this dose represents an ethanol dose at which the adolescent mice did not develop sensitization, while the adult mice developed sensitization. The BEC was measured in adolescents and adults on day 1 (representing acute ethanol treatment) at 10, 60, and 180 min post-ethanol treatment. The 10-min time point was examined to assess the BEC at a time point corresponding to the end of the locomotor behavior session, while the 60 and 180-min time points were examined to assess the clearance of ethanol from the blood. The BEC was also measured in adolescents and adults on day 11 (corresponding to the sensitization test day) to examine any group differences in ethanol clearance after repeated ethanol treatment.

The three-way RM ANOVA of BEC revealed a significant main effect of the between-subject factor treatment day [$F(1, 21)=40.88$; $p<0.001$], a significant main effect of the within-subject factor time post-ethanol injection [$F(2, 42)=586.47$; $p<0.001$], a significant time × age interaction [$F(2, 42)= 3.69$; $p<0.05$], and a significant time × day interaction [$F(2, 42)= 12.32$; $p<0.001$]. To assess whether differences in BEC on day 1 or 11 were responsible for differences

in locomotor activity of adolescents and adults, the BEC data were analyzed separately for days 1 and 11.

A two-way RM ANOVA of BEC on day 1 of ethanol 2.0 g/kg treatment showed a main effect of age [$F(1,6)= 14.54$; $p=0.008$], a main effect of time (minutes) post-ethanol administration [$F(2,12)= 345.43$; $p<0.001$], and a significant interaction [$F(2,11)= 15.09$; $p<0.001$]. Within the adolescents and adults, the post hoc Tukey test revealed that the BEC at 60 min was significantly less than the BEC at 10 min, while the BEC at 180 min was significantly less than the BEC at 60 min (Fig. 4a; $p<0.002$). These data are indicative of ethanol clearance from the blood. Within the time points, the adolescents were significantly different than the adults only at 60 min post-ethanol injection ($p<0.001$). These data indicate that the adolescents had cleared more ethanol from the blood than the adults at 60 min after ethanol 2.0 g/kg administration on day 1.

The two-way RM ANOVA on day 11 revealed no significant main effect of age [$F(1, 5)=0.08$; $p=0.79$], a significant main effect of time post-ethanol injection [$F(2, 10)=184.06$; $p<0.001$], and no significant interaction [$F(2,10)=0.21$; $p=0.81$]. For all mice, the BEC at 60 min was significantly less than the BEC at 10 min, while the BEC at 180 min was significantly less than the BEC at 60 min (Fig. 4b; $p<0.005$), indicating ethanol clearance from the blood over time.

To examine the development of metabolic tolerance in the mice, the BEC at each time point was compared between days 1 and 11. The two-way ANOVA showed a significant main effect of day [$F(1, 27)= 50.25$; $p<0.001$], a significant main effect of time [$F(2, 49)= 582.54$; $p<0.001$], and a significant interaction [$F(2, 49)= 14.59$; $p< 0.001$]. Post hoc Tukey tests indicated that the BEC at 10 and 60 min post ethanol injection differed on day 1 and day 11 ($p<0.001$). These data are indicative of metabolic tolerance to ethanol following repeated administration of ethanol, which has been shown previously in adult and adolescent rats (Chester et al. 2005; Silvers et al. 2003; Varlinskaya and Spear 2007).

Overall, these data show that the adolescent mice clear more ethanol from the blood than adult mice following acute administration of ethanol 2.0 g/kg, but this effect diminishes following repeated administration of ethanol. All of the mice show lower BEC to ethanol following repeated ethanol administration, indicating the development of metabolic tolerance. Importantly, adolescent and adult mice have equivalent BEC at the 10-min time point, which corresponds to the time of the locomotor session during which the age groups display differential ethanol-induced locomotor activity.

Discussion

Adolescence is a time period marked by an increase in risk-taking behavior, which has been shown to lead to experimentation with drugs of abuse such as ethanol (Spear 2000). The effects of ethanol on the maturing adolescent brain are not fully characterized at this time. Studies have shown that adolescent rodents are more or less sensitive than adults to ethanol, depending on the behavior being measured. It has been proposed that these differences in sensitivity might underlie the propensity for ethanol intake during adolescence to lead to alcoholism later in life (see Spear and Varlinskaya 2005). The present study extends the previous findings to ethanol locomotor sensitization and shows that adolescent DBA/2 J mice are less sensitive than adult mice to ethanol-induced neurobehavioral adaptations.

Adolescent DBA/2J mice showed an enhanced locomotor response to acute administration of ethanol 1.5 and 2.0 g/kg compared to the adults. This is in agreement with a recent report in adolescent C57BL/6J mice that showed an increase in locomotor activity after 1.5 g/kg ethanol administration during the first 10 min of testing (Hefner and Holmes 2007). These findings are significant because it has been shown in humans that heavy drinkers are more sensitive to the

acute stimulant effects of ethanol than light drinkers (King et al. 2002).The acute activating effects of ethanol involve numerous neurotransmitter systems, including mesolimbic dopamine signaling, metabotropic and ionotropic glutamate receptors, GABA receptors, and opioid receptors (Blednov et al. 2004; Demarest et al. 1998; Kalivas 1995; Meyer and Phillips 2003; Pastor et al. 2005; Vezina and Kim 1999). The differences observed in the adolescent response to acute ethanol are possibly due to the fact that these neurotransmitter systems are not fully developed in the adolescent (Spear 2000). The undeveloped neurotransmitter systems of the adolescent mice could perhaps be similar to the neurotransmitter systems following sensitization in adult mice. However, this explanation seems unlikely when it is considered that the adolescents showed an enhanced acute response to ethanol 2.5 g/kg, while they also displayed sensitization to this dose.

Repeated administration of ethanol in adult mice leads to an increase in locomotor activation that is markedly greater than the acute locomotor response, known as ethanol sensitization (Phillips et al. 1994). It has been suggested that the neural adaptations which underlie locomotor sensitization might occur in the same brain regions which underlie drug reward and craving (Pierce and Kalivas 1997; Robinson and Berridge 1993; Vanderschuren and Kalivas 2000). Interestingly, ethanol sensitization has not been studied in adolescents. In the present study, the highest dose of ethanol (2.5 g/kg) tested was required to produce locomotor sensitization in the adolescent mice, while multiple ethanol doses (1.5, 2.0, 2.5 g/kg) produced locomotor sensitization in the adult mice. Moreover, the time-course study showed that even with a longer exposure time, the adolescent mice did not develop sensitization to ethanol (2.0 g/kg). These results indicate that adolescent DBA/2J mice are less sensitive to ethanol-induced locomotor sensitization. The finding that adolescents are less sensitive to ethanol sensitization is significant because it has been shown in humans that sons of alcoholics, a group at high risk for developing alcoholism, are differentially sensitive to the physiological effects of ethanol when given repeated ethanol treatments (Newlin and Thomson 1991). Perhaps, blunted sensitivity to the neuroadaptations that occur during the induction of ethanol sensitization in adolescents may be one factor that contributes to the epidemiological observation that adolescent alcohol use is associated with increased risk of abuse in adulthood (Grant and Dawson 1998).

One possible factor that could explain the difference in ethanol sensitization observed in this study is that the blood ethanol concentrations differ between the adolescent and adult groups. For example, adolescent C57BL/6J mice show higher initial BEC than the adults following ethanol (3.0 g/kg) injection but significantly lower BEC by 90 min post-injection, which suggests more rapid ethanol clearance in adolescent mice (Hefner and Holmes 2007). To address this possibility, we evaluated BEC in adolescent and adult mice following injection of the dose of ethanol (2.0 g/kg) that produced differential age-dependent locomotor sensitization. The results show no differences in BEC between the age groups at the 10-min time point either on day 1 or on day 11. This time point is critical because it corresponds to the length of the locomotor session during which the age-groups display differential locomotor activity, indicating dissociation between the BEC and locomotor activity. The results also extend previous findings in adolescent C57BL/6J mice and Sprague-Dawley rats by showing that adolescent DBA/2J mice clear acute ethanol 2.0 g/kg from the blood faster than adults at 60 min post-ethanol administration (Hefner and Holmes 2007; Little et al. 1996). On day 11 of the experiment, when adult mice show locomotor sensitization but adolescent mice do not, no differences in BEC between the age groups are apparent at any time point. These data indicate dissociation between BEC and locomotor sensitization. These data suggest that the age-dependent differential sensitization to ethanol (2.0 g/kg) observed in the present study cannot be attributed to differential BEC.

Another possible explanation for the differential sensitization observed in adolescent mice in this study is that the increased acute response to ethanol 1.5 and 2.0 g/kg affected sensitization. That is, the enhanced acute response on day 1 prevented an increase in locomotor activity from occurring on day 11. However, previous studies have shown that both the neural mechanism and genetic correlates of acute locomotor activation are unrelated to those that underlie ethanol sensitization (Broadbent et al. 1995; Phillips et al. 1995). Furthermore, no correlation between acute locomotor activity and sensitized locomotor activity was observed in the present study, indicating that the acute response on day 1 to ethanol was not predictive of the sensitized response on day 11. The possibility also exists that the acute response to ethanol was at the ceiling of locomotor activity, so that no further increase in activity could be observed following repeated ethanol administration. This can be examined directly by observing the amount of time the animal was ambulatory in the chamber. In this study, the adolescents were ambulatory on days 1 and 11 for less than 5 min of the total 10 min that they were in the locomotor chamber (data not shown). This indicates that the ceiling of locomotor activity had not been reached during the session, as the mice had greater than 5 min to display enhanced locomotor activity. Together, these data confirm that the acute response to ethanol in the adolescents does not underlie their lack of ethanol sensitization.

Previous studies have suggested that increased sensitivity to locomotor sensitization in adults is a marker for increased likelihood of drug dependence (Robinson and Berridge 1993). One might predict, therefore, that adolescents would be more sensitive to ethanol sensitization based on human studies showing that ethanol intake during adolescence increases the likelihood of alcoholism in adulthood (Grant 1998). However, adolescent rodents are known to respond differently to ethanol than adults (see “Introduction”), which means predicted response patterns in adult rodents may not apply to adolescent rodents. In the present study, adolescents were found to be less sensitive to ethanol sensitization, which corresponds to a previous study showing that adolescents were less sensitive to the sedative properties of ethanol (Silveri and Spear 1998). Interestingly, adolescents are more sensitive to ethanol’s inhibition of *N*-methyl-D-aspartate (NMDA)-mediated excitation and long-term potentiation (Swartzwelder et al. 1995a, b). The NMDA receptor antagonist MK-801 has been shown to block ethanol sensitization at higher doses in DBA/2J adult mice (Broadbent and Weitemier 1999; Meyer and Phillips 2003). One possible explanation for the lack of ethanol sensitization seen in adolescent mice is that over the course of the development of sensitization, ethanol is more potently inhibiting the NMDA receptor, which effectively attenuates sensitization. However, at the higher ethanol dose of 2.5 g/kg, the adolescents develop sensitization, which makes this explanation unlikely.

Overall, this is the first study to examine ethanol sensitization in adolescents, and the findings show that adolescent DBA/2J mice are less sensitive to ethanol sensitization than adult mice. This effect is not due to the enhanced acute locomotor response to ethanol in the adolescents or to differences in BEC. These data suggest that blunted sensitivity to ethanol-induced neurobehavioral adaptations during adolescence may be one factor that contributes to increased risk of abuse in adulthood.

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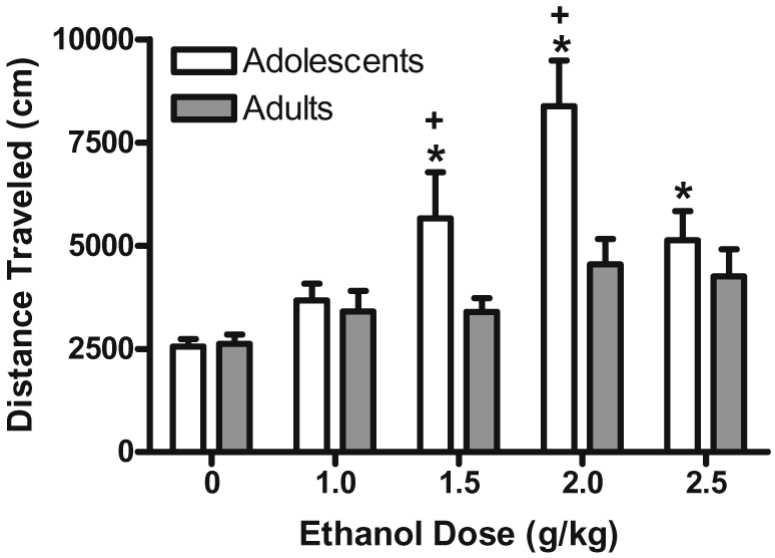

Fig. 1.

Acute locomotor response to ethanol. DBA/2J adolescent (*open bars*) and adult (*filled bars*) locomotor response (distance traveled, in centimeters, mean±SEM) to administration of ethanol (0-2.5 g/kg) during the 10-min session. *Asterisk* indicates significant increase in distance traveled compared to ethanol 0 g/kg, $p<0.05$. *Plus sign* indicates significant increase in distance traveled compared to adult mice, $p<0.05$

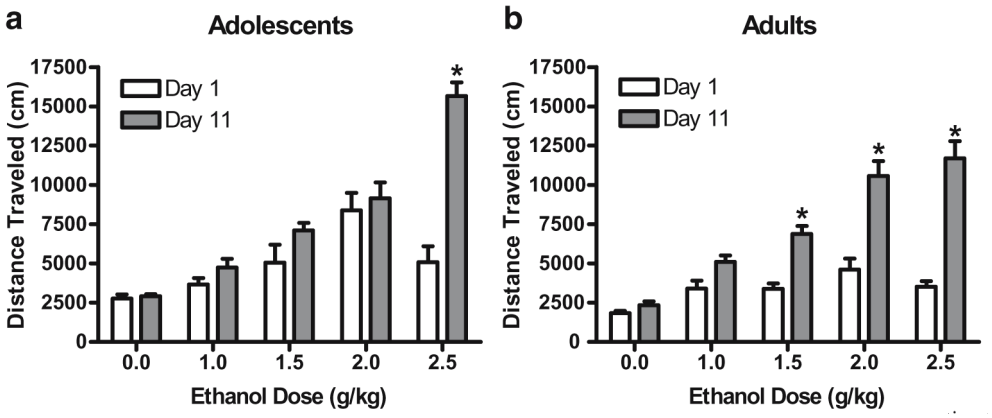

Fig. 2.

Ethanol sensitization: dose response. **a** Adolescent mice locomotor response (distance traveled, in centimeters, mean±SEM) during 10 min test sessions on days 1 and 11 following administration of ethanol (0-2.5 g/kg). *Asterisk* indicates significant increase in distance traveled on day 11 compared to day 1, $p<0.05$. **b** Adult mice locomotor response (distance traveled, in centimeters, mean±SEM) during 10 min test sessions on days 1 and 11 following administration of ethanol (0-2.5 g/kg). *Asterisk* indicates significant increase in distance traveled on day 11 compared to day 1, $p<0.05$

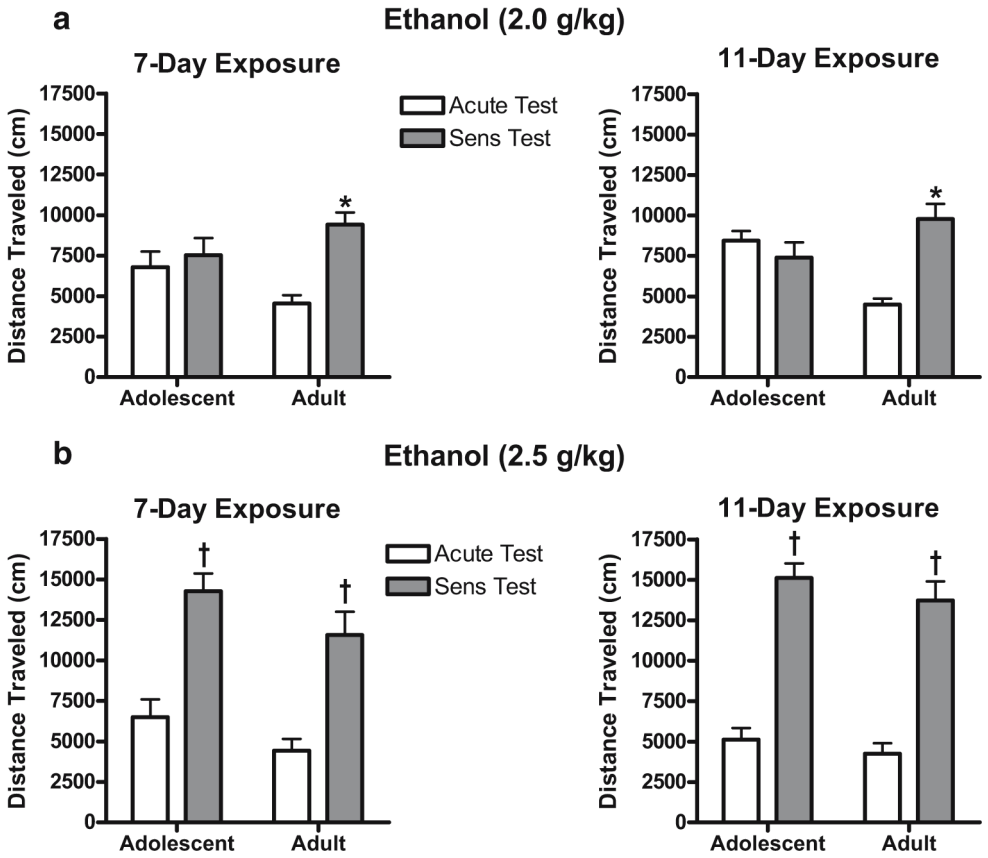

Fig. 3.

Ethanol sensitization: time course. **a** Adolescent and adult locomotor response (distance traveled, in centimeters, mean±SEM) following administration of ethanol 2.0 g/kg during 10 min test sessions on day 1 (acute test; *open bars*) and the final day (7 or 11; sensitization test; *filled bars*). *Asterisk* indicates significant increase in distance traveled compared to day 1, $p<0.05$. **b** Adolescent and adult locomotor response (distance traveled, in centimeters, mean± SEM) following administration of ethanol 2.5 g/kg during 10 min test sessions on day 1 (acute test; *open bars*) and the final day (7 or 11; sensitization test; *filled bars*). *Asterisk* indicates significant increase in distance traveled compared to day 1, $p<0.05$. *Dagger* indicates an overall significant increase from acute test for the two ages combined, $p<0.05$

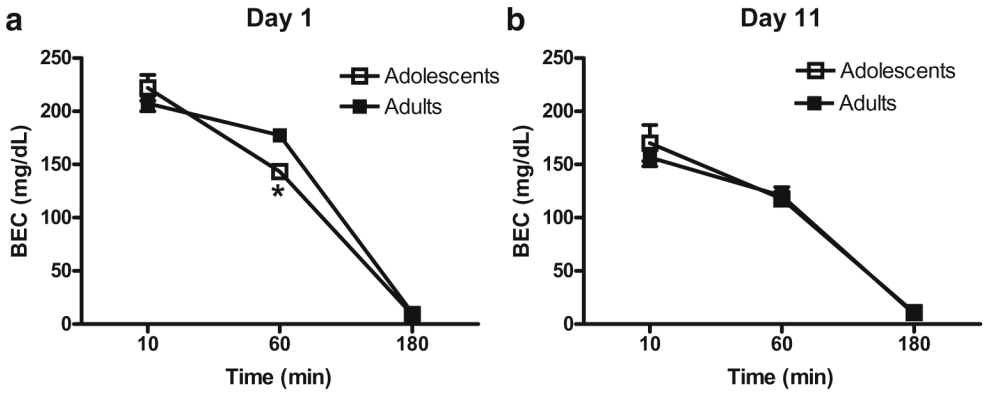

Fig. 4.

Blood ethanol clearance. **a** BEC (mg/dl, mean±SEM) in adolescent and adult DBA/2J mice on day 1, 10, 60, and 180 min following administration of ethanol 2.0 g/kg. **b** BEC (mg/dl, mean ±SEM) in adolescent and adult DBA/2J mice on day 11, 10, 60, and 180 min following administration of ethanol 2.0 g/kg. *Asterisk* indicates significant difference between age groups, $p<0.05$