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Ontogeny of ethanol-induced motor impairment following acute ethanol: Assessment via the negative geotaxis reflex in adolescent and adult rats

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ABSTRACT

Adolescent rats have been observed to be less sensitive than adults to a number of ethanol effects that may serve as feedback cues to reduce further ethanol intake. Among these findings are a few reports of attenuated sensitivities of adolescents to ethanol-induced motor impairment. The purpose of the present study was to further explore potential age-related differences in ethanol-induced motor impairment in both male and female adolescent (postnatal day [P]28–32), and adult (P68–72) Sprague–Dawley rats using an inclined plane assessment of the negative geotaxis reflex. Adult males displayed significant motor impairment at 1.5 g/kg, whereas adolescent males required higher doses, showing significant motor impairment only at doses of 2.25 g/kg ethanol or greater. Intoxicated practice did not significantly influence level of motor impairment at either age. When female rats of both ages were separately analyzed in terms of their response to ethanol, a dose of 1.5 g/kg ethanol was found to significantly impair adults, whereas adolescent females showed significant motor impairment when challenged with 2.25 g/kg but not 1.5 g/kg ethanol. Yet when the 1.5 g/kg data of females at the two ages were directly compared, no significant age difference was seen at this dose. These data document an attenuated sensitivity of adolescent relative to adult rats to the motor impairing effects of ethanol using a stationary inclined plane test, an effect particularly robust in male animals, and demonstrates the utility of this test for assessment of motor coordination in adolescent and adult rats.

Ethanol use is prevalent among adolescents in the United States, with up to as many as 39% of eighth graders, 62% of 10th graders and 72% of 12th graders, reporting some alcohol use in their lifetime (Johnston et al., 2008). According to the recent Monitoring the Future survey results, 44% of 12th graders reported using alcohol in the 30 days prior to the survey, 55% reported having been drunk at least once and 26% reported having consumed five or more drinks in a row during the previous two weeks. How ethanol consumption during adolescence may later affect behavioral and neuronal development as well as its impact on future ethanol use patterns remains to be fully understood. Using an animal model of adolescence, we have found that adolescent rats often differ in their responsiveness to ethanol when compared to adults. Studies from our laboratory have found adolescent rats often voluntarily consume higher amounts of both sweetened and unsweetened ethanol relative to their adult counterparts (Brunell and Spear, 2005; Doremus et al., 2005; Vetter et al., 2007), suggesting that biological factors may contribute to age differences in ethanol consumption. Indeed, adolescence is a time of marked developmental transformations in the brains of both human and nonhuman animals (see Spear, 2000, 2009, for review). These alterations include changes in N-methyl-D-aspartate (NMDA) receptor subunit expression (e.g., Insel et al., 1990) and maturation of $GABA_A$ receptor systems (e.g., Gambarana et al., 1991); there are also ontogenetic changes in the expression of acute tolerance (Varlinskaya and Spear, 2006a), all of which may contribute to age-specific levels of intake and sensitivity to ethanol.

Developmental research in our laboratory and others have shown repeatedly that adolescent animals differ in their responsiveness to acute ethanol effects when compared to adults, although the nature of these ontogenetic differences varies with the ethanol effect under investigation. For instance, adolescent rats have been reported to be more sensitive than adults to the acute effects of ethanol on spatial learning (Markwiese et al., 1998), as well as to the facilitation of social behavior (e.g., Varlinskaya and Spear, 2002, 2006b), while being less sensitive to sedative (Little et al., 1996; Moy et al., 1998; Silveri and Spear, 1998), social inhibitory (e.g., Varlinskaya and Spear, 2004a, 2006a), and aversive (Vetter-O'Hagen et al., 2009) effects of ethanol that may normally serve as cues to limit further ethanol intake. Although little studied, the motor impairing effects of ethanol may be another consequence of ethanol to which adolescents are relatively resistant to when compared with adults (White et al., 2002). Resistance to ethanol disruption of motor coordination, along with the previously mentioned adolescent-typical insensitivities and expression of acute tolerance (Varlinskaya and Spear, 2006a), may

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allow for heavy ethanol use during adolescence potentially putting a number of individuals at risk for future ethanol use disorders.

In rodents, motor coordination has been assessed using a variety of behavioral tasks such as the rotor-rod (e.g., Peris et al., 1997), tilting plane (e.g., Khanna et al., 1994; Tampier and Quintanilla, 2003; White et al., 2002), stationary dowel rod (e.g., Reyes et al., 1993), swim test (e.g., Silveri and Spear, 2001), and moving belt test (e.g., Lê et al., 1989; Lê and Kalant, 1992). These tasks can present challenges for assessing ontogenetic differences in motor impairment, including difficulties in equating baseline motor performance across age (e.g., rotorod task; Spear et al., unpublished observations) or in finding a dose of ethanol that both impaired adolescent and pre-adolescent animals while still allowing adults to perform the task (e.g., swim runway task; Silveri and Spear, 2001). Intoxicated practice, which has been found to augment motor behavior, also plays a major role in influencing performance on other tasks, such as the moving belt task (Lê and Kalant, 1992). Moreover, with the tilting plane task, body weight has been shown to exert a major influence on the angle at which subjects slide on the task, an issue that can complicate interpretation of developmental studies (White et al., 2002).

The purpose of the present study was to extend previous findings of age-related differences in ethanol-induced motor impairment seen with the tilting plane task in adolescent and adult male rats (White et al., 2002), using another motor performance task (the negative geotaxis test). Experiment 1 was designed to test the efficacy using a negative geotaxis reflex test with a fixed inclined plane to examine motor coordination in adolescent and adult rats and the sensitivity of this reflex to disruption by acute ethanol at both ages. Furthermore, using a stationary inclined plane to assess motor performance, we also examined in Experiment 2 whether intoxicated practice or differences in body weight contributed to the age effects observed in Experiment 1 at one or both ages. For this study, the results from Experiment 1 were used to choose an ethanol dose at each age that induced comparable turn latencies at 10 and 30 min post-injection in adult and adolescent male rats.

Lastly, although there are reports of sex differences in ethanol intake that begin in adolescence and continue into adulthood in humans (Witt, 2007) as well as in laboratory animals (Lancaster et al., 1996), few studies have been conducted to explore the extent to which males and females differ in their responsiveness to ethanol. Basic research studies that have examined sex differences have found adult females to be less responsive than adult males to a number of ethanol effects, including ethanol-induced sedation, ethanol-induced social impairment, and anxiogenic effects induced by acute withdrawal (Silveri and Spear, 1998, 1999; Varlinskaya and Spear, 2004b, 2006b). These sex differences found in adults, however, were not evident in adolescent animals of either sex. Thus, males and females show differential sensitivities to ethanol across ontogeny. Experiment 3 was designed to examine whether adolescent and adult female rats differ in their sensitivity to the motor impairing effects of ethanol when tested using the same doses found to produce significant ethanol-induced motor impairment in same-aged adolescent and adult male rats in Experiment 1.

1. Materials and methods

1.1. General methods

1.1.1. Subjects

Adolescent (P28–32) and adult (P68–70) Sprague–Dawley rats used in these experiments were bred and reared in our colony at Binghamton University. On the day after birth (P1), litters were culled to 8 to 10 pups, with 6 animals of one sex and 4 animals of the other being retained whenever possible. Animals were weaned at P21, housed in same-sex littermate pairs, and maintained in a vivarium at Binghamton University on a 14-/10-h light/dark cycle with food and water available ad libitum. Animals were semi-randomly assigned to the experimental groups with the constraint that no more than one subject from a given litter was assigned to a particular treatment condition. Eight to 9 animals were assigned to each experimental group, with each animal tested on only one test day and under one dose condition. Rats used in these experiments were maintained and treated in accordance 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.

1.1.2. Drugs

Ethanol was prepared by dilution in 0.9% NaCl to a concentration of 18.9% (v/v). Ethanol was administered by the intraperitoneal route (i.p.) in volumes adjusted according to the animal's body weight for each dose administered. In each study, the control group at each age was injected with the vehicle (0.9% NaCl) alone at a volume equivalent to that of the highest dose of ethanol used at that age. Solutions were administered at room temperature.

1.1.3. Blood ethanol determination

Immediately following testing, a tail blood sample was collected into a heparinized tube and frozen at −80 °C until analysis of blood ethanol concentration (BEC). Samples were assessed for BEC via headspace gas chromatography using a Hewlett Packard (HP) 5890 series II Gas Chromatograph (Wilmington, DE). At the time of assay, blood samples were thawed and 25-μl aliquots were placed in airtight vials. Vials were placed in a HP 7694E Auto-sampler, which heated each individual vial for 8 min, and then extracted and injected a 1.0 ml sample of the gas headspace into the HP 5890 series Gas 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.

1.1.4. Apparatus

Motor coordination was assessed on an inclined plane. Each inclined plane consisted of a rectangular Plexiglas platform (45.7 cm × 61 cm) painted black and fixed at a 70° angle from horizontal. Each apparatus was covered with a wire mesh screen fixed 0.6 cm above the surface via metal screws. The wire mesh surface was 50.8 cm long, and had a turning width adjusted for each age based on age differences in crown-rump lengths (P28–P32: 15.2 cm wide; P68–P72: 24.1 cm wide). The plane was positioned at the edge of a table with a 73.7 cm drop to provide additional motivation for the animal to avoid moving downward on the plane. A piece of 15.2 cm thick foam was placed under the table's edge to prevent injury to any animal falling from the apparatus.

1.1.5. Testing procedure

Subjects were tested for motor coordination by placing each rat head downward on the stationary inclined plane and determining its latency to rotate 180°. This negative geotaxis reaction is stimulated by the abnormal position of the head and body, initiated by vestibular and postural systems, and requires organized motor movement for successful completion (Adams et al., 1985). Placing the plane at the edge of a table incorporates a cliff aversion reflex into the task as well. Both reflexes are unlearned responses to basic stimuli (e.g., gravitational cues and visual depth perception) and as such require no training, allowing each subject to remain naïve to the test procedure prior to test day.

On each trial, the subject was placed head downward on the inclined plane and its latency to turn 180° to an upright position was recorded in seconds. Each animal was allowed a maximum of 30 s on the apparatus to complete the task. If the rat fell off the plane, the rat was considered to have failed the task and a maximum score of 30 s was assigned. Animals were initially tested prior to the administration

of ethanol or saline to provide baseline data. If a subject fell off during the initial baseline test, it was given one or two additional attempts as needed to complete the turn without falling. Any subject who failed the task on the third trial was not used in these studies (adolescents: $n=1$; adults: $n=3$).

Following baseline measurements, subjects were immediately injected with either ethanol or saline and their motor coordination assessed at a number of predetermined time points post-injection, with a single test trial given at each test point. Between trials, animals were placed individually in holding cages. All sessions were conducted in the presence of a white noise generator to attenuate external noise during testing. During test sessions, the behavior of each animal was recorded by video camera located at the same height and with a frontal view 91.4 cm from the test apparatus. Latency data were determined later from the video records.

1.1.6. Data analysis

Data were analyzed via repeated measures ANOVA. Mauchley's sphericity test for repeated measures designs was performed to test for violations of homogeneity. Epsilon adjustments for non-sphericity were performed using the Greenhouse–Geisser's epsilon test. Dunnett's post-hoc tests were used for comparing differences from a single control group (e.g. dose effects vs. saline), with Fisher's LSD post-hoc tests used for other post-hoc comparisons. Spearman's *R*-tests were used to correlate body weight with behavioral data. A significance level of $p \leq 0.05$ was used for all analyses and comparisons.

1.2. Experiment 1

1.2.1. Methods

A total of 58 adolescent male rats and 32 adult male rats were used in this experiment. Adults were tested following administration of one of four doses of ethanol (0, 1.0, 1.25, 1.5 g/kg i.p.), while the dose range for adolescents was broadened (0, 1.0, 1.25, 1.5, 2.0, 2.25 and 2.5 g/kg i.p.) to determine a dose that induced a comparable level of ethanol-induced motor impairment to that seen following the highest ethanol challenge dose given to adults (1.5 g/kg). Motor coordination was assessed in each animal at baseline as well as 10, 30, and 60 min post-injection.

Due to differences in the range of ethanol doses used at each age, dose–response data for this experiment were analyzed separately at each age.

1.3. Experiment 2

1.3.1. Methods

This experiment explored potential age differences in intoxicated practice effects using a 2 (age) × 2 (number of test trials) factorial design ($n=16$ at each age). For testing, animals at the two ages were given doses of ethanol shown in Experiment 1 to induce equivalent levels of functional impairment at each age (i.e., adolescents: 2.25 g/kg; adults 1.5 g/kg). For each age group, same-aged littermates were semi-randomly assigned to one of two testing groups (with or without intoxicated practice). Group 1 was tested only at 60 min post-ethanol, whereas Group 2 was tested at 10, 30, and 60 min following ethanol injection.

1.4. Experiment 3

1.4.1. Methods

A total of 24 adolescent female rats and 16 adult female rats were used for this experiment. Females at the two ages were given doses of ethanol shown in Experiment 1 to induce equivalent levels of functional impairment in males at these two ages (i.e., adolescents: 2.25 g/kg; adults: 1.5 g/kg). Adolescent females were also tested following administration of the 1.5 g/kg dose to determent whether they, like their male counterparts, would show similar insensitivity to this lower dose of ethanol. Adult females were not tested at the higher dose given preliminary data suggesting that they would be too intoxicated to perform the task at this dose. Thus, doses used for the testing of adolescents were 0 (saline), 1.5, and 2.25 g/kg ethanol i.p., whereas adults were tested following administration of saline or 1.5 g/kg ethanol, with motor coordination assessed at baseline as well as 10, 30, and 60 min post-injection.

Adult and adolescent dose–response data for this experiment were analyzed separately due to differences in number of ethanol doses examined at each age.

2. Results

2.1. Experiment 1

2.1.1. Adult males

A 4 (dose) × 4 (test time: baseline, 10, 30, and 60 min post-injection) ANOVA of the adult data revealed a significant main effect of dose [$F(3,28)=5.70$, $p<0.01$], time [$F(3,84)=7.42$, $p<0.001$], and their interaction [$F(9,84)=2.82$, $p<0.01$]. Adult animals exhibited significant ethanol-induced motor impairment at doses of 1.25 g/kg (at 10 min post-injection), and 1.5 g/kg (at both the 10 and 30 min tests) when compared with saline control animals (see Fig. 1). BECs 60 min post-injection showed that both of the doses of ethanol that produced significant ethanol-induced motor impairment produced BECs in the binge drinking range (NIAAA, 2004) [(mean BEC mg/dl ± SEM) 1.25 g/kg: 102.43 ± 15.17; 1.5 g/kg: 117.21 ± 7.60].

2.1.2. Adolescent males

The 7 (dose) × 4 (test time) ANOVA of the adolescent animals also revealed significant main effects of dose [$F(6,51)=8.41$, $p<0.001$], time [$F(3,153)=3.72$, $p<0.05$], and their interaction [$F(18,153)=2.30$, $p<0.01$]. As seen in Fig. 2, significant ethanol-induced motor impairment was seen in adolescent animals at the 10 and 30 min tests following 2.25 g/kg and at all time points (10, 30, and 60 min) following 2.5 g/kg. Analysis of BECs collected 60 min following 2.25 g/kg and 2.5 g/kg ethanol revealed BECs well above those necessary to be defined as binge-level ethanol exposure (i.e. BECs of ≥80 mg%: 2.25 g/kg—177.20 ± 7.37; 2.5 g/kg—216.73 ± 45.02).

2.1.3. Adolescent vs. adult males at 1.5 g/kg

The results of these analyses conducted separately at each age revealed notable age differences in the effective doses for ethanol-

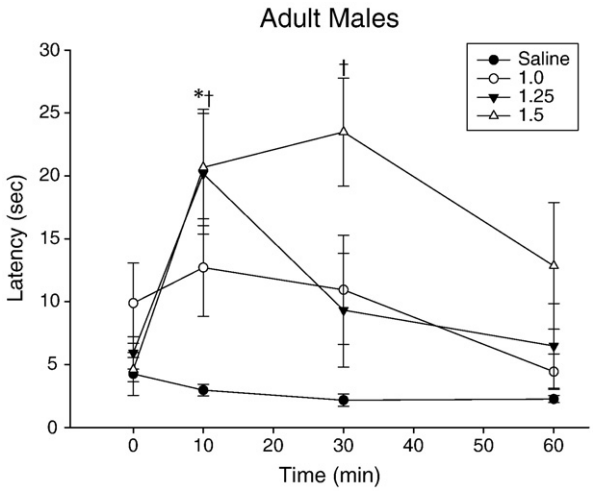

Fig. 1. Adult male animals exhibited significant ethanol-induced motor impairment at doses of 1.25 g/kg (at 10 min post-injection; * $p \leq 0.05$), and 1.5 g/kg (at both the 10 and 30 min tests; † $p \leq 0.05$) when compared with saline control animals.

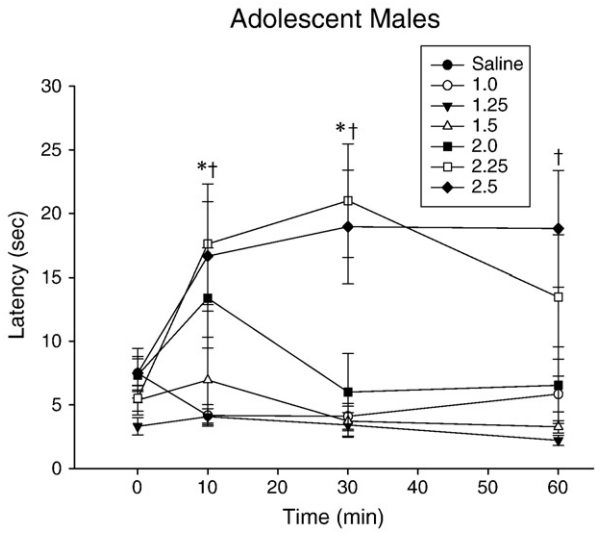

Fig. 2. Significant ethanol-induced motor impairment was seen in adolescent male animals at the 10 and 30 min tests following 2.25 g/kg (* $p \leq 0.05$) and at all time points (10, 30, and 60 min) following 2.5 g/kg († $p \leq 0.05$).

induced motor impairment. To confirm this apparent age difference in sensitivity to the motor impairing effects of ethanol, latency data from adults and adolescents were compared at 1.5 g/kg ethanol, the highest dose of ethanol tested in adults, and a dose sufficient to induce substantial impairment at that age. The results of this 2 (age) × 2 (drug: saline vs. 1.5 g/kg) × 4 (test time) ANOVA showed significant main effects of all variables and their interactions, including the 3-way interaction of age × drug × time [$F(3,84) = 5.50$, $p < .01$]. Significant ethanol-induced motor impairment was seen in adult animals at the 10, 30, and 60 min test intervals whereas adolescents did not exhibit significant ethanol-induced motor impairment at any time point following 1.5 g/kg ethanol (see Fig. 3). Not surprisingly, adults demonstrated significantly longer turn latencies than adolescents at all time intervals following this ethanol challenge dose.

2.1.4. Correlations

Given that body weight can influence behavioral performance on motor tasks, correlations between body weight and latency scores were examined. Correlations were determined for each post-administration interval at the ethanol dose that produced similar ethanol-induced motor impairment in males at each age (adolescents: 2.25 g/kg; adults: 1.5 g/kg); correlations for the saline control groups were also included (see Table 1). These correlational analyses revealed no significant relationship between body weight [mean body weight in grams ± SEM for adolescents: 120.31 ± 4.11; adults: 401.41 ± 6.61] and latencies at any time point following saline or ethanol injection at either age.

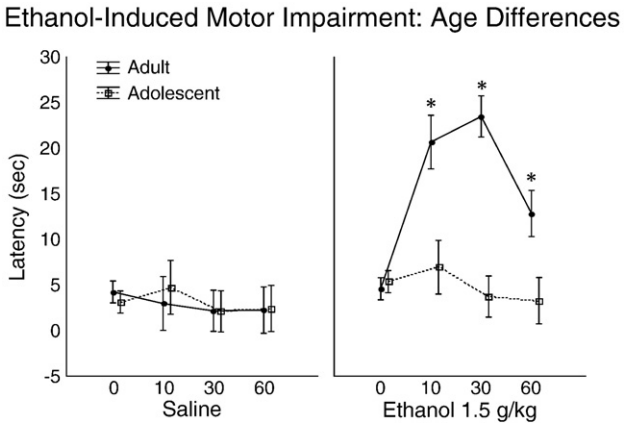

Fig. 3. Significant ethanol-induced motor impairment was seen in adult animals at the 10, 30, and 60 min test intervals following 1.5 g/kg ethanol, whereas adolescents did not exhibit impairment at any interval with this same dose. Adults demonstrated significantly longer turn latencies than adolescents at all time intervals following this ethanol challenge dose. Asterisks indicate significant differences from the saline control group at that test interval, as well as significant difference from ethanol-injected adolescent animals at that same time interval (* $p \leq 0.05$).

2.2. Experiment 2

The data were analyzed via a 2 (number of test trials: 1 vs. 3) × 2 (test time: baseline, 60 min) repeated measures ANOVA at each age. The only significant findings emerging in these ANOVAs were main effects of time (adolescents: [$F(1,14) = 12.66$, $p < 0.01$]; adults: [$F(1,14) = 6.00$, $p < 0.05$]), with turn latencies longer at the 60 min interval than at baseline at both ages shown in Fig 4. The ANOVA conducted on BECs revealed no effect of number of test trials at either age [(mean BEC mg/dl ± SEM) adolescents: 1 trial: 190.22 mg/dl ± 8.85; 3 trials: 172.45 ± 21.93; adults: 1 trial: 129.81 ± 5.66; 3 trials: 132.92 ± 8.09].

Thus, under these test circumstances, there was no evidence that intoxicated practice facilitated performance on the inclined plane in either adult or adolescent male rats using a test dose at each age (1.5 g/kg and 2.25 g/kg ethanol, respectively) that induced comparable levels of impairment.

2.3. Experiment 3

2.3.1. Adult females

A 2 (dose) × 4 (test time) ANOVA of the adult female data revealed a significant main effect of dose [$F(1,14) = 5.53$, $p < 0.05$], time [$F(3,42) = 2.78$, $p \leq 0.05$], and their interaction [$F(3,42) = 2.92$, $p < 0.05$]. Adult animals exhibited significant ethanol-induced motor impairment at the 10 and 30 min tests post-ethanol injection (1.5 g/kg) when compared with saline control animals (see Fig. 5, right panel).

2.3.2. Adolescent females

The 3 (dose) × 4 (test time) ANOVA of the adolescent female animals revealed a significant main effect of dose [$F(2, 21) = 8.52$, $p < 0.01$], time [$F(3, 63) = 3.10$, $p < 0.05$], and their interaction [$F(6, 63) = 2.45$, $p < 0.05$]. Significant ethanol-induced motor impairment was seen in adolescent animals at all time points (10, 30, and 60 min) following 2.25 g/kg, but not following administration of the lower dose (see Fig 5, left panel).

2.3.3. Adolescent vs. adult females at 1.5 g/kg

The results of the analyses conducted separately at each age revealed an age difference in the effective dose for ethanol-induced motor

Table 1
Correlations between body weight and latency score.

	Post-injection		
	10 min	30 min	60 min
Adolescents (saline)			
Male	0.38	−0.56	0.02
Female	−0.15	−0.27	0.22
Adolescents (2.25 g/kg)			
Male	−0.32	−0.27	0.22
Female	−0.35	0.08	0.06
Adults (saline)			
Male	0.55	−0.14	−0.23
Female	0.47	−0.29	0.07
Adults (1.5 g/kg)			
Male	0.19	0.16	0.28
Female	0.74*	0.18	0.17

Correlations of body weight and latency data at all time points (10, 30, and 60 min tests) following saline or ethanol injection in adolescent and adult animals. Data are expressed as the mean ± SEM. Asterisks indicate significance (* $p \leq 0.05$).

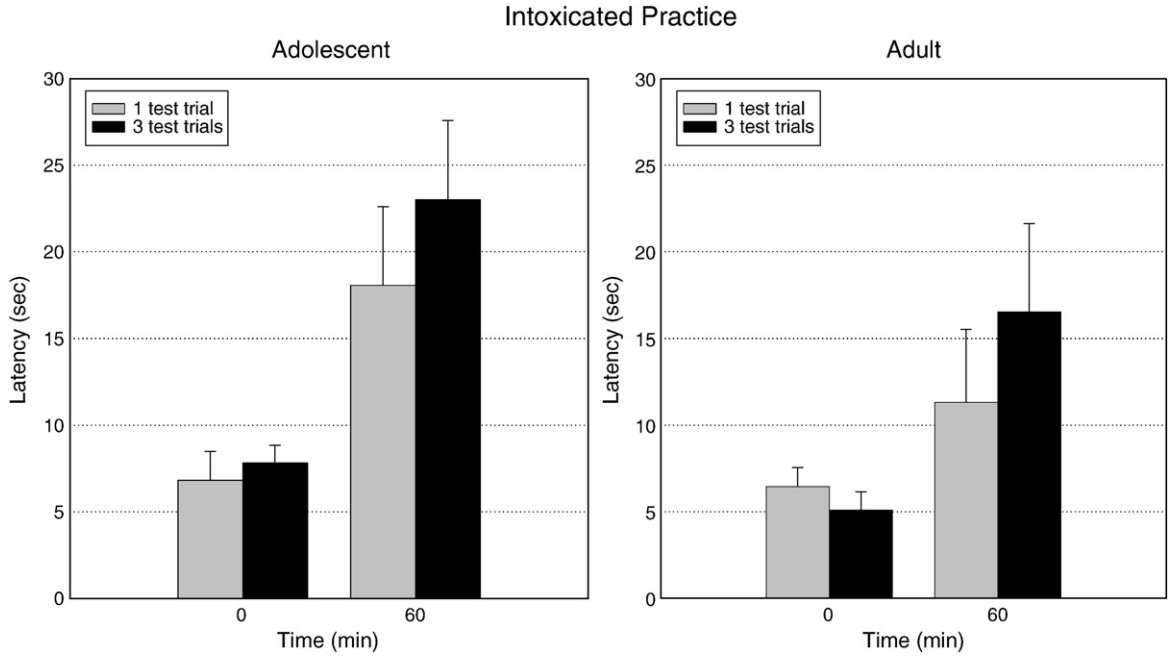

Fig. 4. There was no evidence that intoxicated practice facilitated performance on the inclined plane in either adult or adolescent male rats using a test dose at each age (1.5 g/kg and 2.25 g/kg ethanol, respectively) that induced comparable levels of motor impairment. All data are expressed as the mean ± SEM.

impairment, with adolescent females showing ethanol-induced motor impairment following 2.25 g/kg, but not 1.5 g/kg ethanol, whereas adult females were significantly impaired at the 1.5 g/kg challenge dose. However, when data from adolescent and adult females were directly compared at the 1.5 g/kg dose, using a 2 (age) × 2 (drug: saline vs. 1.5 g/kg) × 4 (test time) ANOVA, no main effect or interaction involving age emerged. The only significant effects that emerged were main effects of drug [$F(1, 28) = 6.56$, $p < 0.05$], and time [$F(3, 84) = 4.37$, $p < 0.01$] and their interaction [$F(3, 84) = 4.01$, $p \leq 0.01$]. The ANOVA conducted on BECs likewise did not reveal an effect of age [(mean BEC mg/dl ± SEM) adolescents: 117.45 ± 5.29, adults: 129.28 ± 10.68].

2.3.4. Correlations

Correlations between female body weight and turn latencies were calculated in a manner analogous to that used for males in Experiment 1. For adolescent females, body weights [mean body weight in grams ± SEM: 110.13 ± 4.02] did not correlate with turn latencies at any time point when tested following saline or 2.25 g/kg ethanol. For adult females, body weight [mean ± SEM: 253.81 g. ± 5.48] was significantly correlated with turn latencies only at the 10 min test time following 1.5 g/kg ethanol (see Table 1). This effect may be spurious in that no such association was seen in these adult females at the other test intervals following ethanol or saline injection.

3. Discussion

This study provides additional evidence that adolescents are less sensitive to the motor impairing effects of acute ethanol when compared to adults. In the present study, when motor coordination was assessed using a stationary inclined plane, adult male rats displayed significant motor impairment following doses of 1.25 g/kg and 1.5 g/kg, whereas adolescent males required higher doses, showing significant motor impairment only following challenge with 2.25 g/kg and 2.5 g/kg ethanol. Unlike age differences that

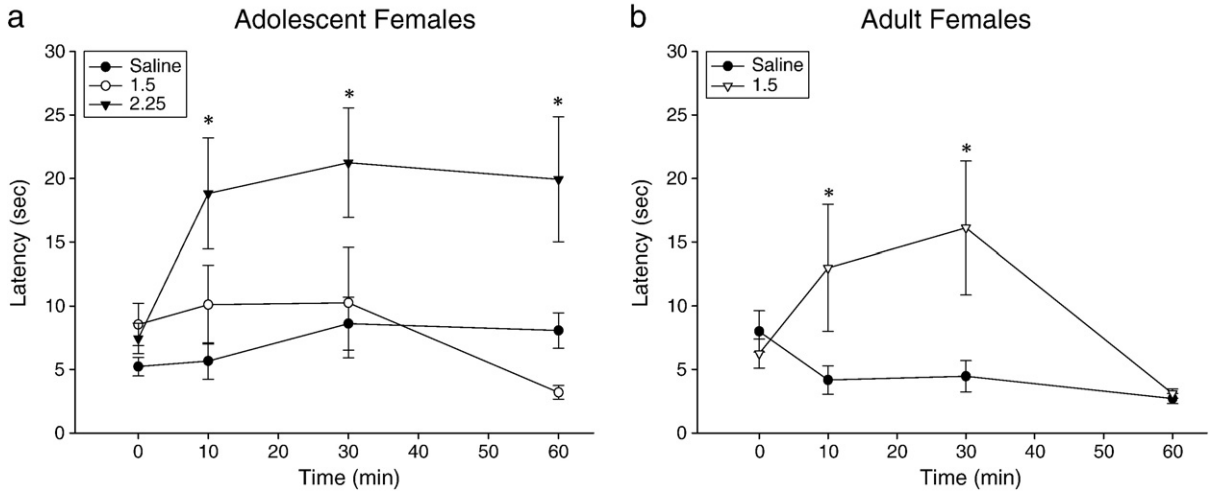

Fig. 5. (a) Significant ethanol-induced motor impairment was seen in adolescent female animals at all time points (10, 30, and 60 min) following 2.25 g/kg, but not following administration of the lower dose of 1.5 g/kg ethanol. (b) Adult females exhibited significant ethanol-induced motor impairment at the 10 and 30 min tests post-ethanol injection (1.5 g/kg) when compared with saline control animals. Asterisks indicate significant differences from the saline control group at that test interval (*$p \leq 0.05$).

were observed between males in Experiment 1 following challenge with 1.5 g/kg ethanol, results from Experiment 3 revealed mixed findings as to whether adolescent and adult female rats differ significantly in their sensitivity to ethanol-induced motor impairment on the negative geotaxis task. On the one hand, separate analyses at each age revealed that adult females were disrupted by a dose of 1.5 g/kg ethanol, whereas motor performance of adolescents was significantly impaired at a dose of 2.25 g/kg, but not following 1.5 g/kg ethanol. On the other hand, these data were not sufficiently robust to reveal an age difference at the 1.5 g/kg dose when the analysis focused on animals of both ages challenged with saline or this dose of ethanol. It is possible that adolescent females in the saline group may have had slightly higher latency scores than adult females at 30 and 60 min post-ethanol injection, which could have contributed to the lack of sensitivity of adolescents to this dose of ethanol (1.5 g/kg). However, saline latency data were very similar in females at both ages at 10 min post-injection, a time interval during which adults showed a significant response to the 1.5 g/kg challenge, whereas adolescents did not. Thus, differing baseline latencies alone do not appear to drive the age difference observed. Taken together, these analyses suggest somewhat less robust age differences in motor impairment on this task between adolescent and adult female rats relative to those seen in their male counterparts.

Research conducted in our laboratory which have included animals of both sex have sometimes observed females to exhibit fewer age differences in the aversive effects of ethanol as well as in ethanol consumption levels relative to males (Varlinskaya and Spear, 2004b; Vetter-O'Hagen et al., 2009). For instance, when hangover effects were indexed via overall social activity, behavioral signs of acute ethanol withdrawal were found to be more severe in adult than adolescent males and than females at either age, with no age effect seen in females (Varlinskaya and Spear, 2004b). Additionally, when examining ethanol intake in a limited access situation, significant age differences were observed between adolescent and adult males, with adolescents consuming approximately 3 times as much ethanol than their adult counterparts, while no significant differences were observed in intake between adolescent and adult females (Vetter-O'Hagen et al., 2009). Sex differences in the impact of age on measures of alcohol sensitivity and intake are not always evident, however. For instance, significant differences in ethanol intake were observed between adolescent and adult rats of both sexes under continuous access conditions (Doremus et al., 2005). Likewise, no differences have been observed between males and females in the magnitude of ethanol-induced social facilitation typically seen in adolescent, but not adult animals (e.g., Varlinskaya and Spear, 2006b).

A factor known to augment behavioral performance on motor tasks such as the moving belt task and tilt-plane task (Lê et al., 1989; Khanna et al., 1994) is repeated practice while intoxicated. However, results from Experiment 2 showed that when adolescent and adult male animas were tested repeatedly post-ethanol, their motor performance was not significantly different from the animals that were tested only once while intoxicated. Therefore, intoxicated practice did not influence the level of motor impairment on the inclined plane for either age in the male animals tested here.

Another potential contributor to the findings observed is body weight, given that adult male rats clearly outweighed the adolescent animals as well as same-aged females used in this study. Indeed, previous studies with the tilting plane have found that body weight can influence the angle at which a subject begins to slide on the task (Tampier and Quintanilla, 2003; White et al., 2002). Analyses of correlations between body weight and latency scores at each age and sex revealed no consistent evidence for body weight influences on motor performance in this task in either saline or ethanol treated animals of either age or sex.

The finding that adolescents are less sensitive than adults to the motor impairing effects of ethanol is consistent with other data from our laboratory and others where adolescent animals have often been found to differ in their responsiveness to acute ethanol effects when compared to adults. Neural alterations that are occurring throughout the adolescent period may be influencing the responsiveness of adolescents to ethanol in a way that differs from adult response patterns. For instance, the relative insensitivity of adolescent rats to ethanol-induced motor impairment may be in part a consequence of developmental over-expression of NMDA receptors and age-specific subunit compositions. NMDA receptors, particularly those containing NR2A and NR2B subunits (Allgaier, 2002), are among the highest affinity ethanol targets in the brain and play a functional role in neuronal excitability (Nagy, 2004), cognitive function (Malhotra et al., 1996), and motor coordination (Sanchez-Perez et al., 2005) as well as in mediating the intoxicating effects of ethanol (Kumari and Ticku, 2000). Densities of NMDA receptor sites increase substantially across ontogeny in rats to reach levels at P21 that are above those seen in adult rats (Pruss, 1993); developmental changes in subunit expression also occurs, with NR2B receptor subunit predominance early in the life (Portera-Cailliau et al., 1996) switching to a more even distribution of NR2A and NR2B receptors in many brain regions by adulthood (Sheng et al., 1994). These developmental differences in NMDA receptor expression and subunit composition, via altering NMDA receptor function and sensitivity, may contribute to the differential sensitivity of adolescents to ethanol.

Other adolescent-specific alterations, such as age-related differences in the development of within session tolerance (acute tolerance) to ethanol may further influence the responsiveness of adolescents to acute ethanol effects. Indeed, studies examining the ontogeny of acute tolerance have found young rats through adolescence to exhibit substantially more acute tolerance than adults (Silveri and Spear, 1998; Varlinskaya and Spear, 2006a) when using measures such as ethanol-induced social facilitation, social inhibition (Varlinskaya and Spear, 2004a, 2006a), and recovery of the righting reflex (Silveri and Spear, 2004). Whether acute tolerance to ethanol-induced motor impairment contributed to the age differences observed here is as of yet unknown, but under current investigation.

The results of this study add further to the overwhelming evidence that adolescents differ in their responsiveness to ethanol when compared to adults. Adolescent animals were found to be less sensitive than adults to the motor impairing effects of acute ethanol using an inclined plane assessment of the negative geotaxis reflex, an effect that was particularly pronounced among male animals. Additional work is necessary to explore further the mechanisms contributing to the age differences in sensitivity to the motor impairing effects of ethanol. This work presents a new motor impairment task that can be used for assessing ontogenetic differences in motor impairment, with both adolescent and adult rats showing comparable baseline response latencies, along with age-specific, dose-dependent impairment to ethanol that was unaffected by variables such as body weight and intoxicated practice.

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References

Adams J, Buelke-Sam J, Kimmel CA, Nelson CJ, Reiter LW, Sobotka TJ, et al. Collaborative Behavioral Teratology Study: protocol design and testing procedures. Neurobehav Toxicol Teratol 1985;7:579–86.

Allgaier C. Ethanol sensitivity of NMDA receptors. Neurochem Int 2002;41:377–82.

Brunell SC, Spear LP. Effect of stress on the voluntary intake of a sweetened ethanol solution in pair-housed adolescent and adult rats. Alcohol Clin Exp Res 2005;29:1641–53.

Doremus TL, Brunell SC, Pottayil R, Spear LP. Factors influencing elevated ethanol consumption in adolescent relative to adult rats. Alcohol Clin Exp Res 2005;29:1796–808.

Gambarana C, Beattie CE, Rodriquez ZR, Siegel RE. Region-specific expression of messenger RNAs encoding GABAA receptor subunits in the developing rat brain. Neuroscience 1991;45:423–32.
Insel TR, Miller LP, Gelhard RE. The ontogeny of excitatory amino acid receptors in rat forebrain: I. N-methyl-d-aspartate and quisqualate receptors. Neuroscience 1990;35:31–43.
Johnston LD, O'Malley PM, Bachman JG, Schulenberg JE. Monitoring the future national survey results on drug use, 1975–2007. Secondary school students (NIH Publication No. 08-6418A)Bethesda, MD: National Institute on Drug Abuse; 2008.
Khanna JM, Morato GS, Chau A, Shah G, Kalant H. Effect of NMDA antagonists on rapid and chronic tolerance to ethanol: importance of intoxicated practice. Pharmacol Biochem Behav 1994;48:755–63.
Kumari M, Ticku MK. Regulation of NMDA receptors by ethanol. Prog Drug Res 2000;54: 152–89.
Lancaster FE, Brown TD, Coker KL, Elliott JA, Wren SB. Sex differences in alcohol preference and drinking patterns emerge during the early postpubertal period in Sprague–Dawley rats. Alcohol Clin Exp Res 1996;20:1043–9.
Lê AD, Kalant H. Influence of intoxicated practice on the development of acute tolerance to the motor impairment effect of ethanol. Psychopharmacology 1992;106:572–6.
Lê AD, Kalant H, Khanna JM. Roles of intoxicated practice in the development of ethanol tolerance. Psychopharmacology 1989;99:366–70.
Little PJ, Kuhn CM, Wilson WA, Swartzwelder HS. Differential effects of ethanol in adolescent and adult rats. Alcohol Clin Exp Res 1996;20:1346–51.
Malhotra AK, Pinals DA, Weingartner H, Sirocco K, Missar CD, Pickar D, et al. NMDA receptor function and human cognition: the effects of ketamine in healthy volunteers. Neuropsychopharmacology 1996;14:301–7.
Markwiese BJ, Acheson SK, Levin ED, Wilson WA, Swartzwelder HS. Differential effects of ethanol on memory in adolescent and adult rats. Alcohol Clin Exp Res 1998;22: 416–21.
Moy SS, Duncan GE, Knapp DJ, Breese GR. Sensitivity to ethanol across development in rats: comparison to [3H]zolpidem binding. Alcohol Clin Exp Res 1998;22:1485–92.
Nagy J. The NR2B subtype of NMDA receptor: a potential target for the treatment of alcohol dependence. Curr Drug Targets CNS Neurol Disord 2004;3:169–79.
NIAAA. National Institute on Alcohol Abuse and Alcoholism council approves definition of binge drinking. NIAAA newsletter 3; 2004. Bethesda, MD.
Peris J, Sealey SA, Jung BJ, Gridley KE. Simultaneous cocaine exposure abolishes ethanol tolerance. Behav Pharmacol 1997;8:319–30.
Portera-Cailliau C, Price DL, Martin LJ. N-methyl-D-aspartate receptor proteins NR2A and NR2B are differentially distributed in the developing rat central nervous system as revealed by subunit-specific antibodies. J Neurochem 1996;66:692–700.
Pruss R. Receptors for glutamate and other excitatory amino acids: a cause for excitement in nervous system development. In: Zagon IS, McLaughlin PJ, editors. Receptors in the developing nervous system: neurotransmitters, vol. 2. New York: Chapman and Hall; 1993. p. 141–62.
Reyes E, Duran E, Switzer SH. Effects of in utero administration of alcohol on alcohol sensitivity in adult rats. Pharmacol Biochem Behav 1993;44:307–12.
Sanchez-Perez A, Llansola M, Cauli O, Felipo V. Modulation of NMDA receptors in the cerebellum. II. Signaling pathways and physiological modulators regulating NMDA receptor function. Cerebellum 2005;4:162–70.
Sheng M, Cummings J, Roldan LA, Jan YN, Jan LY. Changing subunit composition of heteromeric NMDA receptors during development of rat cortex. Nature 1994;368: 144–7.
Silveri MM, Spear LP. Decreased sensitivity to the hypnotic effects of ethanol early in ontogeny. Alcohol Clin Exp Res 1998;22:670–6.
Silveri MM, Spear LP. Ontogeny of rapid tolerance to the hypnotic effects of ethanol. Alcohol Clin Exp Res 1999;23:1180–4.
Silveri MM, Spear LP. Acute, rapid and chronic tolerance during ontogeny: observations when equating ethanol perturbation across age. Alcohol Clin Exp Res 2001;25: 1301–8.
Silveri MM, Spear LP. The effects of NMDA and GABAA pharmacological manipulations on acute and rapid tolerance to ethanol during ontogeny. Alcohol Clin Exp Res 2004;28:884–94.
Spear LP. The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 2000;24:417–63.
Spear LP. The behavioral neuroscience of adolescence. New York: W.W. Norton & Company Inc; 2009.
Tampier L, Quintanilla MI. Involvement of brain ethanol metabolism on acute tolerance development and on ethanol consumption in alcohol-drinker (UChB) and non-drinker (UChA) rats. Addict Biol 2003;8:279–86.
Varlinskaya EI, Spear LP. Acute effects of ethanol on social behavior of adolescent and adult rats: role of familiarity of the test situation. Alcohol Clin Exp Res 2002;26: 1502–11.
Varlinskaya EI, Spear LP. Changes in sensitivity to ethanol-induced social facilitation and social inhibition from early to late adolescence. Ann N Y Acad Sci 2004a;1021: 459–61.
Varlinskaya EI, Spear LP. Acute ethanol withdrawal (hangover) and social behavior in adolescent and adult male and female Sprague–Dawley rats. Alcohol Clin Exp Res 2004b;28:40–50.
Varlinskaya EI, Spear LP. Ontogeny of acute tolerance to ethanol-induced social inhibition in Sprague–Dawley rats. Alcohol Clin Exp Res 2006a;30:1833–44.
Varlinskaya EI, Spear LP. Differences in the social consequences of ethanol emerge during the course of adolescence in rats: social facilitation, social inhibition, and anxiolysis. Dev Psychobiol 2006b;48:146–61.
Vetter CS, Doremus-Fitzwater TL, Spear LP. Time-course of elevated ethanol intake in adolescent relative to adult rats under continuous, voluntary-access conditions. Alcohol Clin Exp Res 2007;31:1159–68.
Vetter-O'Hagen CS, Varlinskaya EI, Spear LP. Sex differences in ethanol intake and sensitivity to aversive effects during adolescence and adulthood. Alcohol Alcohol 2009;44:547–54.
White AM, Truesdale MC, Bae JG, Ahmad S, Wilson WA, Best PJ, et al. Differential effects of ethanol on motor coordination in adolescent and adult rats. Pharmacol Biochem Behav 2002;73:673–7.
Witt ED. Puberty, hormones, and sex differences in alcohol abuse and dependence. Neurotoxicol Teratol 2007;29:81–95.