Published in final edited form as:

Alcohol. 2014 June ; 48(4): 353–360. doi:10.1016/j.alcohol.2014.02.003.

Effect of Sub-Chronic Intermittent Ethanol Exposure on Spatial Learning and Ethanol Sensitivity in Adolescent and Adult Rats

H. S. Swartzwelder^{1,2}, A. Hogan², M-Louise Risher^{1,2}, Rita A. Swartzwelder², Wilkie A. Wilson^{2,3}, and Shawn K. Acheson^{1,2}

¹Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, North Carolina

²Neurobiology Research Laboratory, Durham Veterans Affairs Medical Center, Durham, North Carolina

³Social Sciences Research Institute, Duke University, Durham, North Carolina

Abstract

It has become clear that adolescence is a period of distinct responsiveness to the acute effects of ethanol on learning and other cognitive functions. However, the effects of repeated intermittent ethanol exposure during adolescence on learning and cognition are less well studied, and other effects of repeated ethanol exposure such as withdrawal and chronic tolerance complicate such experiments. Moreover, few studies have compared the effects of repeated ethanol exposure during adolescence and adulthood, and they have yielded mixed outcomes that may be related to methodological differences and/or secondary effects of ethanol on behavioral performance. One emerging question is whether relatively brief intermittent ethanol exposure (i.e., sub-chronic exposure) during adolescence or adulthood might alter learning at a time after exposure when chronic tolerance would be expected, and whether tolerance to the cognitive effects of ethanol might influence the effect of ethanol on learning at that time. To address this, male adolescent and adult rats were pre-treated with sub-chronic daily ethanol (five doses [4.0 g/kg, i.p.] or saline at 24-h intervals, across 5 days). Two days after the last pre-exposure, spatial learning was assessed on 4 consecutive days using the Morris water maze. Half of the animals from each treatment cell received ethanol (2.0 g/kg, i.p.) 30 min prior to each testing session and half of the animals received saline. Ethanol pre-exposure altered water maze performance in adolescent animals but not in adults, and acute ethanol exposure impaired learning in animals of both ages independent of pre-exposure condition. There was no evidence of cognitive tolerance in animals of either age group. These results indicate that a relatively short period of intermittent ethanol exposure during adolescence, but not adulthood, alters baseline water maze performance shortly after pre-exposure and does not induce cognitive tolerance to the effects of ethanol in either age group.

Corresponding Author: H. S. Swartzwelder, Ph.D., Durham VAMC – Research (151), 508 Fulton Street, Durham, NC 27705, Telephone: +1 919 971 0964, Fax: +1 919 286 6811, hss@duke.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

adolescent; learning; water maze; tolerance; alcohol

Introduction

The enduring effects of repeated ethanol exposure during adolescence have become a topic of intense investigation in recent years. Depending upon the dose and duration of chronic exposure, and the dependent measures addressed, different features of the long-term effects of such exposure may be explored. For example, when dependent measures are taken within the first 24–48 h after the termination of chronic exposure, the outcomes may reflect withdrawal (Acheson et al., 1999). When measures are taken after the acute withdrawal phase, but within a period of days to weeks, the outcomes may reflect chronic tolerance, particularly when acute ethanol challenges are part of the experimental paradigm (Silveri and Spear, 2001). Beyond that time frame it is likely that enduring changes reflect long-term, perhaps permanent effects on CNS function or responsiveness (White et al., 2002).

The periods after ethanol exposure during which tolerance is expressed are particularly important because tolerance contributes to ethanol abuse and addiction. For example, tolerance to some ethanol effects contributes to the escalation of drinking and thereby increases the risks associated with acute intoxication as well as the likelihood that dependence will develop. Because drinking during adolescence places individuals at elevated risk for the development of alcohol abuse (Grant and Dawson, 1998), it is important to understand whether and how tolerance develops during adolescence and whether this process differs in any meaningful ways from how tolerance develops in adulthood.

Studies of ethanol tolerance during adolescence have generally used rather brief exposure paradigms, sufficient for tolerance induction but not presumed to be sufficient to cause long-term or permanent change in CNS function. The number of exposures to ethanol and the time at which tolerance is assessed after ethanol exposure are important in determining the type of tolerance being manifested. There is general agreement on the usefulness of identifying three distinct phases or manifestations of tolerance (see (Kalant, 1993). *Acute tolerance* occurs after a single dose of ethanol and is manifested during the time when that dose remains pharmacologically active. On a slightly longer time frame, *rapid tolerance* is manifested in response to a second dose of ethanol presented within 24 h of an initial, single dose. *Chronic tolerance* is manifested as a diminished response to an acute ethanol challenge persisting for days or weeks after termination of a period of chronic ethanol exposure. Clearly, there are many parameters related to the measurement of tolerance, including the number and dosage of pre-exposures, the time after pre-exposure that the challenge is presented, the dose of the challenge itself, and the dependent measure being assessed at the time of the challenge. The common feature, however, is a diminished response to acute ethanol, and because diminished response to ethanol has both safety and clinical implications it is important to understand whether these processes differ between adolescence and adulthood.

Early experiments that made direct comparisons between tolerance development in adolescent and adult rats indicated that adolescents developed both acute (Silveri & Spear, 1998) and chronic (Swartzwelder et al., 1998) tolerance more readily than did adults when using ethanol-induced sleep time (Silveri and Spear, 1998; Swartzwelder et al., 1998) or body temperature regulation (Swartzwelder et al., 1998) as the dependent measures. It is notable, however, that when lower amounts of ethanol were given, chronic tolerance to the sedative effects of ethanol was observed in adult rats but not in adolescents (Broadwater et al., 2011). Adolescent rats have also been shown to be less likely than adults to manifest rapid tolerance to the hypnotic effects of ethanol 24 h after a single ethanol dose (Silveri and Spear, 1999). Interestingly, when motor performance (swimming) was used as the dependent measure and the efficacy of acute ethanol was indexed to the brain ethanol levels achieved by adolescent and adult rats, respectively, adolescent and adult animals did not differ with respect to the development of rapid or chronic tolerance, and adolescent animals appeared somewhat less likely to develop acute tolerance than did adults (Silveri and Spear, 2001). These findings differed notably from the previous studies, and the inconsistency is likely to be related to the fact that different doses of ethanol were used across age groups in the effort to equalize motor impairment. More recently, when ethanol-induced social inhibition was used as a dependent measure to reflect tolerance in rats, adolescents manifested acute tolerance to a low dose of ethanol (1.0 g/kg, i.p.) but adults did not (Varlinskaya and Spear, 2006). Clearly, there are substantive differences in tolerance development between adolescent and adult animals. However, whether adults or adolescents are more susceptible to tolerance depends upon the type of tolerance being assessed, the dependent measures being used, and the specific ethanol doses utilized to induce and/or display tolerance.

Because spatial learning and memory have been shown to be more sensitive to disruption by both acute (Markwiese et al., 1998) and chronic (Sircar and Sircar, 2005) ethanol exposure in adolescent rats, compared to adults, it is important to understand whether adolescents and adults differ in the acquisition of “cognitive” tolerance in learning paradigms. In a clever series of experiments, Silvers et al. (Silvers et al., 2003; Silvers et al., 2006) showed that chronic intermittent ethanol (CIE) exposure across the 20-day period of adolescence in the rat markedly reduced the ability of ethanol to impair spatial learning in the Morris water maze 24 h after the last in the series of chronic ethanol doses. This indicates that adolescent animals develop significant cognitive tolerance to ethanol after an extended, but intermittent exposure paradigm. Interestingly, this same group showed that a different chronic intermittent exposure paradigm, using exposure to ethanol vapor over a shorter (4-day) period during adolescence, did not result in cognitive tolerance (Van Skike et al., 2012). This raises the question of whether longer exposure periods might be necessary to induce cognitive tolerance in adolescent animals, or if the pharmacokinetic differences between vapor exposure and parenteral administration might account for the differences in observed tolerance. It is also important to understand whether adolescents and adults differ with respect to development of cognitive tolerance. Because the studies cited above did not include adult animals, it is unclear whether adolescence represents a distinct period of vulnerability or resistance to the development of cognitive tolerance.

Finally, it is not clear whether relatively brief chronic intermittent ethanol exposure results in baseline differences in subsequent learning. The studies cited above (Sillers et al., 2003; Silvers et al., 2006) found no differences in baseline spatial learning, but they trained adolescent animals during, not after, the chronic intermittent dosing sequence. In contrast, Sircar & Sircar (Sircar and Sircar, 2005) found greater spatial learning and memory impairments in adolescents than in adults after five daily doses of ethanol, but the dosing interval was twice as frequent as in the studies by Silvers et al. (Sillers et al., 2003; Silvers et al., 2006).

To begin to address some of these questions, the present study was designed to assess whether sub-chronic exposure to ethanol during adolescence or adulthood in the rat altered performance in the spatial version of the Morris water maze task at a time after signs of withdrawal would be expected to have subsided but during which tolerance could be present. In addition, we sought to determine if the sub-chronic ethanol exposure would alter the effects of acute ethanol challenge on learning in this paradigm, possibly reflecting cognitive tolerance and enabling a direct comparison between the effects of sub-chronic intermittent ethanol exposure in adolescent and adult animals.

Methods

Animals and Treatments

All of the procedures used in this study were conducted in accordance with the guidelines of the American Association for the Accreditation of Laboratory Animal Care and the National Research Council's Guide for Care and Use of Laboratory Animals and were approved by the Durham VAMC and the Duke University Institutional Animal Care and Use Committees.

Forty-eight male Long-Evans hooded rats (Charles River, USA) were used in this study. One animal died during the course of the experiments. Twenty-four rats arrived on postnatal day 25 (adolescent) and 24 arrived on postnatal day 65 (adult). They were double-housed with *ad libitum* access to food and water (the cage mate of the animal that died was replaced with a male rat of the same age in order to maintain double housing). They were allowed to acclimatize for 5 days in the vivarium on a 12-h light/12-h dark cycle (lights off at 6:00 PM) prior to beginning CIE/saline (VWR, Suwanee, GA, USA) administration. All dosing and behavioral testing was done during the light cycle, and both age groups were tested conjointly.

Ethanol or saline pre-exposures were initiated when the rats were at postnatal day 30 or 70, representing adolescence and adulthood, respectively. All animals were exposed to a sub-chronic daily pre-exposure regimen. Half of the animals in each age group ($n = 12$) received five doses of 4 g/kg ethanol (16.9% v/v in saline at 30 mL/kg) and the other half in each age group received isovolumetric saline, administered by intraperitoneal injection (i.p.) at 24-h intervals across 5 days. This dose was chosen for the pre-exposure phase because it has been shown to induce loss of righting reflex in about 50% of both adolescent and adult rats (although it does result in longer sleep times in adults) (Little, Kuhn, Wilson, & Swartzwelder, 1996). Thus, it represents a high dose with generally similar behavioral

efficacy in adolescents and adults. Forty-eight hours after the last ethanol or saline pre-exposure, spatial learning training was initiated in the Morris water maze. Prior to the initiation of training, the treatment groups were subdivided so that half of the animals ($n = 6$) in each pre-exposure cell received a 2.0 g/kg (12.7% v/v in saline at 20 mL/kg) dose of ethanol 30 min prior to each water maze testing session and half of the animals in each pre-exposure cell received isovolumetric saline (i.p.). This acute dose was chosen because our previous studies have shown that it results in similar BECs 30–60 min after administration (i.e., during the time of behavioral testing in this study) in adolescent and adult rats (Little et al., 1996). Thus, the experiment was designed to assess the effect of ethanol pre-exposure alone, ethanol challenge during water maze training alone, or their combination on spatial learning acquisition.

Water Maze Training

The water maze tank was constructed of white fiberglass and measured 1.5 m wide and 0.75 m deep. The tank was filled with water ($\sim 22^\circ\text{C}$) until the white plastic platform (9 cm diameter) was submerged ~ 1 cm below the water line. The platform location remained constant throughout the study and was located in the NW quadrant. The thigmotaxis zone was a concentric area consisting of the outermost 23% of the maze field. The inside perimeter of this thigmotaxis zone was 9 cm from the outer perimeter. The non-thigmotaxis zone was the remainder of the maze field. These zones were used to calculate the distance animals swam in thigmotaxis and non-thigmotaxis. ANY-maze software (Stoelting; Wood Dale, IL) and a ceiling-mounted digital camera were used to record and digitize the swim path of each animal on each trial. The room was equipped with numerous distal extra-maze cues to facilitate spatial learning. Each animal was given six trials per day for 4 consecutive days. Animals were placed into the tank facing the wall at one of three locations (NE, SE, and SW). Each starting location was used twice and the order was pseudo-randomized to avoid using the same starting point on two consecutive trials, and the sequence varied across days for each animal. Training trials lasted 60 sec or until the animal located the platform. Animals were given 15 sec on the platform and then allowed to rest for 30 sec in a warm dry towel before the next trial. Trials were run consecutively for each animal on each day. A camera mounted above the maze and ANY-maze tracking software was used to capture and analyze the performance of each subject during each trial. The time to reach the goal platform and distance traveled were both recorded. Because animals are placed randomly in non-platform quadrants of the maze on each trial, the distance from the platform can vary somewhat trial-to-trial. Therefore, we used the measure of Path Efficiency as the primary dependent variable in our analysis of spatial learning. On each trial the ANY-maze software calculated the ‘ideal path’ that the animal could take to reach the submerged platform. The path efficiency measure is the ratio of the actual swim path length to the ideal path for that trial. A path efficiency of 1.0 would represent an exact overlap of the actual path with the ideal path. We also assessed swim speed and thigmotaxis in order to more effectively interpret the effects of ethanol on water maze performance.

Blood Ethanol Levels

To identify possible developmental differences in blood ethanol levels that could influence the behavioral results of this study, blood ethanol concentration (BEC) was measured in a

separate group of animals ($n = 8/\text{treatment cell}$) on Day 1 (first day of the pre-exposure phase) and Day 8 (the first day of behavioral testing). The ethanol-exposure procedures matched those in the behavioral study, and BEC samples were drawn by lancet puncture of the lateral saphenous vein 120 min post-injection on Day 1, and 30 minutes post-injection on Day 8. These delays were chosen to represent approximate peak BEC (Livy, Parnell, & West, 2003) and the time at which behavioral testing was initiated, respectively. Serum was collected from centrifuged blood samples and stored at -80°C . Ethanol concentration was analyzed in duplicate using an Analox GL5 alcohol analyzer (Analox Instruments; Lunenburg, MA) and expressed as mg/dL.

Statistical Analyses

Because of the ambiguity associated with the interpretation of 3- and 4-way interactions within an ANOVA, we analyzed behavioral data from adolescent and adult animals separately. This constrained formal age-related comparisons. However, because the age groups were treated and tested together, differences between the effects of ethanol on adolescent and adult animals were acknowledged. These data were analyzed using repeated-measures analyses of variance (ANOVAs) with Day as the repeated measure and pre-exposure (4.0 g/kg ethanol vs. saline) and acute exposure (2.0 g/kg ethanol vs. saline) as between-subjects variables. Simple main effects analyses were performed in the presence of significant interactions. Dependent measures included path efficiency (actual path length/ideal path length), distance swum in thigmotaxis, and swim speed. The difference between adolescent and adult blood ethanol concentrations on Day 1 was analyzed using Student's t test. ANOVA was used to test the effects of age (adolescent vs. adult) and pre-exposure (ethanol vs. saline) on Day 8.

All analyses were performed with SPSS v18 (IBM, Chicago). The sphericity assumption was tested using Mauchly's W for all repeated measures. Degrees of freedom were adjusted using the Greenhouse-Geisser correction where Mauchly's W was significant ($p < 0.05$) and Lower-bound where Mauchly's W was undefined. Ordinal interactions were followed by tests of simple main effects. Alpha was set at $p = 0.05$ for all analyses. Effect size was reported as partial η^2 (η_p^2).

Results

There were no age-related differences in BEC 120 min post-injection on Day 1 ($t_{(14)} = 0.14$, $p = 0.44$). However, there was an effect of Age ($F_{(1,28)} = 26.16$, $p = 0.001$) and an Age $\times$ Pre-exposure interaction ($F_{(1,28)} = 8.67$, $p = 0.006$) on BEC collected on Day 8 (see Fig. 1). Analyses of simple main effects revealed a Pre-exposure effect in adult animals ($t_{(14)} = 2.75$, $p = 0.009$) but not in adolescent animals ($t_{(14)} = 1.19$, $p = 0.13$).

Spatial learning was evident across training days in the water maze in both adolescent ($F_{(3,66)} = 82.19$, $p < 0.001$; Fig. 2) and adult ($F_{(3,57)} = 84.51$, $p < 0.001$; Fig. 3) animals, and the degree of learning was dependent on acute ethanol exposure in both age groups with ethanol producing impairment in learning relative to saline (Adolescents: $F_{(3,66)} = 8.92$, $p < 0.001$; Adults: $F_{(3,57)} = 25.89$, $p < 0.001$). Among adolescent animals, acute ethanol impaired spatial learning on Days 2, 3, and 4 (all $p < 0.01$) but not Day 1 ($p = 0.16$). In

contrast, acute ethanol impaired spatial learning on all days ($p = 0.001$) in adult animals. Interestingly, acute ethanol exposure in adolescent animals accounted for increasingly more variance across days, from 8% on Day 1 to 50% on Day 4 (see Table 1 for exact statistics), suggesting a change in the animals' sensitivity over time. There was no linear increase in effect size across days among the adult animals (Day 1: 43%, Day 2: 85%, Day 3: 76%, Day 4: 75%).

Swimming in the thigmotaxis zone (i.e., near the outer wall of the water maze) decreased significantly across test days in animals of both ages (Adolescents: $F_{(3,66)} = 104.78$, $p < 0.001$, Fig. 4; Adults: $F_{(3,57)} = 64.39$, $p < 0.001$, Fig. 5). In adolescent animals, the decline in thigmotaxis was dependent on acute exposure to ethanol ($F_{(3,66)} = 6.78$, $p = 0.002$), with acute ethanol exposure producing more thigmotaxis on each test day, relative to controls (all p 's < 0.02). Among adult animals, the decline in thigmotaxis was also dependent on acute ethanol exposure ($F_{(3,57)} = 8.94$, $p = 0.002$), which caused more thigmotaxis among treated animals than was observed in controls on each test day. In contrast to the results in adolescents, however, thigmotaxis in adults was also influenced by ethanol pre-exposure ($F_{(3,57)} = 6.28$, $p = 0.008$). This effect appears to have been driven by differences on Day 1 of testing (Fig. 5), when thigmotaxis is most likely to be observed (Acheson, Moore, Kuhn, Wilson, & Swartzwelder, 2011). It should be noted that adult animals pre-exposed to ethanol were more thigmotactic than saline pre-exposed adult animals, but qualitatively similar to saline and ethanol pre-exposed adolescent animals.

Swim speed also declined across days in both adolescent ($F_{(3,66)} = 68.61$, $p < 0.001$) and adult ($F_{(3,57)} = 16.32$, $p < 0.001$) animals. The decline in adolescent swim speed was dependent on acute ethanol exposure ($F_{(3,66)} = 5.35$, $p = 0.002$), with ethanol-treated adolescents swimming faster than saline-treated animals on Day 3 ($p = 0.002$) and Day 4 ($p = 0.01$), but not Day 1 ($p = 0.88$) or Day 2 ($p = 0.06$). The decline in adult swim speed was dependent on both acute ethanol exposure ($F_{(3,57)} = 12.78$, $p < 0.001$) and ethanol pre-exposure ($F_{(3,57)} = 9.98$, $p < 0.001$). As in adolescents, acute ethanol increased swim speed in adults [increases on Days 2, 3, and 4 (all $p < 0.001$), but not Day 1 ($p = 0.6$)]. Conversely, ethanol pre-exposure in adults increased swim speed on Day 1 ($p = 0.002$), but not Days 2–4 (all $p > 0.44$).

Discussion

We found that there was no evidence of cognitive tolerance to the effects of ethanol challenge on the days on which spatial learning training occurred, in either adolescent or adult animals. Previous studies have been equivocal on the development of such cognitive tolerance and suggest that the parameters of ethanol pre-exposure are important for predicting its development. Moreover, to our knowledge no previous studies have sought to directly compare adolescent and adult animals with respect to cognitive tolerance development. The lack of cognitive tolerance that we observed in adolescent rats is consistent with the findings of Van Skike et al. (2012), who assessed the effect of ethanol challenge on a previously learned spatial task 28 h after termination of a 4-day, intermittent ethanol vapor exposure period. However, other studies, using longer ethanol pre-exposure periods (encompassing the bulk of the adolescent period in the rat) have shown evidence of

cognitive tolerance in spatial learning (Silvers et al., 2003; Silvers et al., 2006). It is possible that the longer pre-exposure period in those studies could account for the development of cognitive tolerance, but it is also possible that differences in spatial learning training could account for the difference. For example, Silvers et al. (2003) trained rats during the ethanol pre-exposure period, on the days between ethanol administrations, and then tested them for cognitive tolerance using a 1-day testing procedure 24 h after the last training session. Thus, by the time of the single ethanol challenge, the procedural components of the spatial learning task were well established. In contrast, in the present study, animals received no training during the pre-exposure period, and the development of spatial learning was assessed over a 4-day acquisition period with no previous maze experience. Again, while it is likely that the difference in the number of pre-exposures accounts for some of the differences we observed, it is also possible that tolerance is more readily observed when measuring the effect of ethanol on procedurally established spatial memory, compared to initial learning. In addition, chronic tolerance is often measured as a shift to the right in the dose-response curve, so our use of only one challenge dose during behavioral training in the present study requires cautious interpretation. Still, the dose we used was chosen because it has been shown to have similar effects on water maze performance in adolescent and adult animals (Markwiese et al., 1998) and because it represents a behaviorally relevant dose but not one that is likely to induce sedation or motor deficits. Another methodological consideration is that the animals in the present study were delivered to the vivarium and allowed 5 days to adapt there before procedures were initiated. There is no standardized approach to adaptation in developmental studies of this type, but this relatively brief period of adaptation could have resulted in a different degree of stress to the animals in this study than they might have experienced had they been bred in-house or delivered at earlier ages. In any case it is notable that the present findings are consistent with the lack of cognitive tolerance recently observed after a relatively brief pre-exposure period in adolescent rats (Van Skike et al., 2012), and the results extend that finding to a direct comparison with parallel pre-exposure in adults.

In recent experiments using a longer ethanol pre-exposure period than in the present study and a 20-day ‘washout’ period after pre-exposure, we have found no evidence of enduring effects of ethanol pre-exposure on baseline spatial learning in either the radial arm maze (Risher et al., 2013) or the Morris water maze (unpublished observations). This was the case whether the pre-exposure occurred in adolescence or adulthood. In this sense the present data are consistent with our recent observations and underscore what appears to be an emerging trend in the literature, namely, that intermittent pre-exposure with ethanol has little consistent effect on subsequent baseline spatial learning though it influences subsequent learning-related responsiveness to acute ethanol, at least when the pre-exposure occurs during adolescence (Risher et al., 2013; White et al., 2000).

One behavioral feature of water maze performance is thigmotaxis, i.e., swimming persistently in close proximity to the outer wall of the maze. For example, a recent study found that the cannabinoid agonist, WIN-55212-2, disrupted spatial learning in the water maze (Acheson et al., 2011). However, the apparent effect on spatial learning was eliminated after controlling for thigmotaxis. Similarly, Sircar & Sircar (2005) described spatial learning deficits in the water maze in adult rats 30 days after daily ethanol pre-

exposure during adolescence, and also found an increase in thigmotaxis. In the present study, we found that acute ethanol exposure during training promoted thigmotaxis in both age groups, despite the fact that adult animals achieved lower BECs on Day 1 of behavioral training (Fig. 1). Importantly, however, ethanol pre-exposure only promoted subsequent thigmotaxis in adult animals, having no effect on thigmotaxis in adolescents, and the increase in thigmotaxis on Day 1 among adults pre-exposed to ethanol was also accompanied by an increase in swim speed on that test day for animals in that treatment cell. It has been suggested that thigmotaxis may reflect anxiety under certain behavioral testing conditions. Thus, it is possible that sub-chronic ethanol pre-exposure gave rise to a greater anxiogenic response upon introduction to the water maze in adults than in adolescents. It is also notable that, among adult animals, the treatment group that manifested the most thigmotaxis on Day 1 of testing was the group that had received both acute exposure and pre-exposure with ethanol (Fig. 5, top panel). This occurred despite the fact that ethanol pre-exposed adults achieved lower BECs than saline pre-exposed adults (Fig. 1). It is also possible, therefore, that sub-chronic ethanol exposure in adults gave rise to a greater anxiogenic response to acute ethanol. These are intriguing possibilities that suggest subtle but important differences in the responsiveness of adolescent and adult animals to relatively brief periods of ethanol exposure. Additional studies using direct age comparisons will be required to explore these possibilities further.

The present results indicate that a brief period of intermittent ethanol exposure during adulthood, but not adolescence, alters performance in the water maze, specifically by promoting thigmotaxis. Moreover, there was no evidence for cognitive tolerance to the acute effects of ethanol in either adolescent or adult animals, expanding the recent findings of Van Skike et al. (2012), and suggesting that relatively long periods of ethanol pre-exposure may be required in order for cognitive tolerance to be observed. These findings also underscore the need for studies of chronic ethanol exposure to include both adolescent and adult treatment groups in order to allow direct age-related comparisons. As the new literature on the effects of repeated ethanol exposure during adolescence has emerged, it has become obvious that experimental parameters such as ethanol pre-exposure paradigms, post-exposure intervals, specific behavioral assessments, and direct adolescent-adult comparison groups are of great importance. The present findings contribute to the understanding of some of these issues and should serve as a valuable backdrop for the design of future studies.

Acknowledgments

This work was supported by the NIAAA grant U01-AA019925-01 (NADIA) to HSS, a VA Senior Research Career Scientist award to HSS, and a VA Career Development Award I01BX007080 to SKA.

References

- Acheson SK, Moore NL, Kuhn CM, Wilson WA, Swartzwelder HS. The synthetic cannabinoid WIN 55212-2 differentially modulates thigmotaxis but not spatial learning in adolescent and adult animals. *Neuroscience Letters*. 2011; 487:411–414. [PubMed: 21055447]
- Acheson SK, Richardson R, Swartzwelder HS. Developmental changes in seizure susceptibility during ethanol withdrawal. *Alcohol*. 1999; 18:23–26. [PubMed: 10386661]

- Broadwater M, Varlinskaya EI, Spear LP. Chronic intermittent ethanol exposure in early adolescent and adult male rats: effects on tolerance, social behavior, and ethanol intake. *Alcoholism: Clinical and Experimental Research*. 2011; 35:1392–1403.
- Grant BF, Dawson DA. Age of onset of drug use and its association with DSM-IV drug abuse and dependence: results from the National Longitudinal Alcohol Epidemiologic Survey. *Journal of Substance Abuse*. 1998; 10:163–173. [PubMed: 9854701]
- Kalant H. Problems in the search for mechanisms of tolerance. *Alcohol and Alcoholism*. 1993; (Supplement, 2):1–8. [PubMed: 7748283]
- Little PJ, Kuhn CM, Wilson WA, Swartzwelder HS. Differential effects of ethanol in adolescent and adult rats. *Alcoholism: Clinical and Experimental Research*. 1996; 20:1346–1351.
- Livy DJ, Parnell SE, West JR. Blood ethanol concentration profiles: a comparison between rats and mice. *Alcohol*. 2003; 29:165–171. [PubMed: 12798972]
- Markwiese BJ, Acheson SK, Levin ED, Wilson WA, Swartzwelder HS. Differential effects of ethanol on memory in adolescent and adult rats. *Alcoholism: Clinical and Experimental Research*. 1998; 22:416–421.
- Risher ML, Fleming RL, Boutros N, Semenova S, Wilson WA, Levin ED, et al. Long-term effects of chronic intermittent ethanol exposure in adolescent and adult rats: radial-arm maze performance and operant food reinforced responding. *PloS one*. 2013; 8:e62940. [PubMed: 23675442]
- Silveri MM, Spear L. Decreased sensitivity to the hypnotic effects of ethanol early in ontogeny. *Alcoholism: Clinical and Experimental Research*. 1998; 22:670–676.
- Silveri MM, Spear LP. Ontogeny of rapid tolerance to the hypnotic effects of ethanol. *Alcoholism: Clinical and Experimental Research*. 1999; 23:1180–1184.
- Silveri MM, Spear LP. Acute, rapid, and chronic tolerance during ontogeny: observations when equating ethanol perturbation across age. *Alcoholism: Clinical and Experimental Research*. 2001; 25:1301–1308.
- Silvers JM, Tokunaga S, Mittleman G, Matthews DB. Chronic intermittent injections of high-dose ethanol during adolescence produce metabolic, hypnotic, and cognitive tolerance in rats. *Alcoholism: Clinical and Experimental Research*. 2003; 27:1606–1612.
- Silvers JM, Tokunaga S, Mittleman G, O'Buckley T, Morrow AL, Matthews DB. Chronic intermittent ethanol exposure during adolescence reduces the effect of ethanol challenge on hippocampal allopregnanolone levels and Morris water maze task performance. *Alcohol*. 2006; 39:151–158. [PubMed: 17127134]
- Sircar R, Sircar D. Adolescent rats exposed to repeated ethanol treatment show lingering behavioral impairments. *Alcoholism: Clinical and Experimental Research*. 2005; 29:1402–1410.
- Swartzwelder HS, Richardson RC, Markwiese-Foerch B, Wilson WA, Little PJ. Developmental differences in the acquisition of tolerance to ethanol. *Alcohol*. 1998; 15:311–314. [PubMed: 9590516]
- Van Skike CE, Novier A, Diaz-Granados JL, Matthews DB. The effect of chronic intermittent ethanol exposure on spatial memory in adolescent rats: the dissociation of metabolic and cognitive tolerances. *Brain Research*. 2012; 1453:34–39. [PubMed: 22464184]
- Varlinskaya EI, Spear LP. Ontogeny of acute tolerance to ethanol-induced social inhibition in Sprague-Dawley rats. *Alcoholism: Clinical and Experimental Research*. 2006; 30:1833–1844.
- White AM, Bae JG, Truesdale MC, Ahmad S, Wilson WA, Swartzwelder HS. Chronic-intermittent ethanol exposure during adolescence prevents normal developmental changes in sensitivity to ethanol-induced motor impairments. *Alcoholism: Clinical and Experimental Research*. 2002; 26:960–968.
- White AM, Ghia AJ, Levin ED, Swartzwelder HS. Binge pattern ethanol exposure in adolescent and adult rats: differential impact on subsequent responsiveness to ethanol. *Alcoholism: Clinical and Experimental Research*. 2000; 24:1251–1256.

Figure 1. Mean ($\pm$ SEM) blood ethanol concentration. Top Panel: 120 min post-injection on Day 1; Bottom Panel: 30 min post-injection on Day 8 (open bars: saline pre-exposure; shaded bars: ethanol pre-exposure). Star symbol represents statistical significance at $p < 0.01$.

Figure 2.

Mean ($\pm$ SEM) swim path efficiency for adolescent rats. Top panel: each of the four pre-exposure by acute treatment cells [saline pre-exposure/acute saline (open circles); saline pre-exposure/acute ethanol (solid circles); ethanol pre-exposure/acute saline (open triangles); ethanol pre-exposure/acute ethanol (closed triangles)]. Lower panels: simple main effects of pre-exposure (left side) and acute treatment (right side); saline: open squares, ethanol: closed squares. Note: some SEM ranges are smaller than the graphic symbols. Star symbols represent statistical significance at $p < 0.01$. Note: Ordinate for top and lower panels are identical.

Figure 3.

Mean ($\pm$ SEM) swim path efficiency for adult rats. Top panel: each of the four pre-exposure by acute treatment cells [saline pre-exposure/acute saline (open circles); saline pre-exposure/acute ethanol (solid circles); ethanol pre-exposure/acute saline (open triangles); ethanol pre-exposure/acute ethanol (closed triangles)]. Lower panels: simple main effects of pre-exposure (left side) and acute treatment (right side); saline: open squares, ethanol: closed squares. Note: some SEM ranges are smaller than the graphic symbols. Star symbols represent statistical significance at $p < 0.001$. Note: Ordinate for top and lower panels are identical.

Figure 4.

Mean ($\pm$ SEM) distance in thigmotaxis zone for adolescent rats. Top panel: each of the four pre-exposure by acute treatment cells [saline pre-exposure/acute saline (open circles); saline pre-exposure/acute ethanol (solid circles); ethanol pre-exposure/acute saline (open triangles); ethanol pre-exposure/acute ethanol (closed triangles)]. Lower panels: simple main effects of pre-exposure (left side) and acute treatment (right side); saline: open squares, ethanol: closed squares. Note: some SEM ranges are smaller than the graphic symbols. Star symbols represent statistical significance at $p < 0.02$. Note: Ordinate for top and lower panels are identical.

Figure 5.

Mean ($\pm$ SEM) distance in thigmotaxis zone for adult rats. Top panel: each of the four pre-exposure by acute treatment cells [saline pre-exposure/acute saline (open circles); saline pre-exposure/acute ethanol (solid circles); ethanol pre-exposure/acute saline (open triangles); ethanol pre-exposure/acute ethanol (closed triangles)]. Lower panels: simple main effects of pre-exposure (left side) and acute treatment (right side); saline: open squares, ethanol: closed squares. Note: Ordinates for top and lower panels are identical; some SEM ranges are smaller than the graphic symbols. Star symbols represent statistical significance at $p < 0.05$.

Table 1

Effect Size (95% Confidence Interval) for Simple Main Effect of Acute Ethanol on Training Days 1 - 4

	Day 1	Day 2	Day 3	Day 4
Adolescents	0.08 (0.00 - 0.31)	0.24 (0.01 - 0.47)	0.45 (0.14 - 0.63)	0.50 (0.19 - 0.67)
Adults	0.43 (0.11 - 0.63)	0.85 (0.69 - 0.90)	0.76 (0.52 - 0.85)	0.75 (0.50 - 0.84)