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Repeated ethanol exposure affects the acquisition of spatial memory in adolescent female rats

Ratna Sircar^{1,2}, Ashim K. Basak¹, and Debashish Sircar²

¹ *Feinstein Institute for Medical Research, Manhasset, NY*

² *Departments of Psychiatry & Behavioral Sciences, Neurology, Pathology Albert Einstein College of Medicine, Bronx, NY 10461*

Abstract

Ethanol has been reported to disrupt spatial learning and memory in adolescent male rats. The present study was undertaken to determine the effects of ethanol on the acquisition of spatial memory in adolescent female rats. Adolescent female rats were subjected to repeated ethanol or saline treatments, and spatial learning was tested in the Morris water maze. For comparison, adult female rats were subjected to similar ethanol treatment and behavioral assessments as for adolescent rats. Ethanol-treated adolescent rats took longer and swam greater distances to find the hidden platform than saline controls. In the probe trial, ethanol-treated adolescent rats showed a trend towards reduced time spent in the target quadrant, and made significantly fewer target location crossings than saline-treated controls. Adult saline-treated control rats did not learn the spatial memory task as well as the adolescent saline-treated rats. Although ethanol in adult rats increased both latency and swim distance to find the platform, in the probe trial there was no difference between ethanol-treated adult rats and age-matched saline controls. Ethanol did not alter swim speed or performance in the cued visual task at either age. Together, these data suggest that ethanol specifically impairs the acquisition of spatial memory in adolescent female rats. Since adult females did not learn the task, ethanol-induced alterations in water maze performance may not reflect true learning and memory dysfunction.

Keywords

Morris water maze; juvenile; adult; age-related; alcohol; learning and memory; brain development; cognition

Introduction

According to the National Institute on Alcohol Abuse and Alcoholism of the 15.1 million individuals in the United States that abuse ethanol or are ethanol-dependent, nearly one third (4.6 million) are women. Human epidemiological studies indicate that after chronic ethanol exposure women show significant behavioral dysfunction [36, see review 24]. Although women generally start to drink later in life and intake per occasion is low, they progress rapidly to ethanol dependence and have greater adverse consequences [17].

Name and mailing address of the corresponding author: Ratna Sircar, Ph.D. The Feinstein Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030, Tel: (516) 562-1068, Fax: (516) 562-1008, Email: E-mail: rsircar@nshs.edu.

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A disturbing trend has recently been noticed among females in that they have started to use and abuse ethanol at much younger ages [17]. Ninth-grade girls consume as much or more ethanol than do ninth-grade boys, and more of them binge drink [14]. This lowering of age for first-use of alcohol in women is of concern since how alcohol affects the still-developing brain in adolescent females remains unclear. Brain regions such as the prefrontal cortex, limbic brain regions, and reward circuitry are known to undergo active development during adolescence [6,11]. Female adolescents with alcohol-use disorder (AUD) have smaller prefrontal cortex volume [21] and show significant white matter abnormalities [7] compared to adolescent males with AUD. Also, evidence from functional imaging studies indicate that female adolescents with AUD show significant brain abnormalities during spatial working memory tasks [5].

Several studies have examined the acute effects of ethanol on cognitive functioning in adult animals, particularly spatial learning and memory [4,20,41]. Adult male mice injected with ethanol 24 h after having being trained in the Morris water maze take longer and swim greater distances to find the hidden platform [3]. Acute ethanol in adult male rats also compromises performance in the radial arm spatial task [38]. But Rossetti et al [28] reported bidirectional modulation of spatial working memory by acute ethanol in adult animal, with improvements at lower doses and impairments at higher doses.

Cognitive deficits following chronic ethanol exposure have been reported in some adult animal studies. Mice made to drink ethanol for 4–8 weeks when tested in the T-maze avoidance task show significant deficits [9]. Savage and coworkers [29] treated male rats with ethanol for 20 weeks followed by withdrawal, and found delay-independent impairment in the delayed nonmatching-to-position task. But Steigawald and Miller [35] reported that chronic ethanol in adult male mice not only did not impair learning in the radial arm maze but actually slightly improved their performance. In a study where rats were exposed to a binge ethanol paradigm followed by withdrawal and then tested in the Morris water maze, ethanol did not affect the acquisition of reference memory or working memory, but these rats required more time to learn the reversal task [25].

Repeated ethanol treatment just prior to daily memory testing in adult male animals has been shown to impair hippocampal dependent learning. Ethanol administration prior to tone-shock pairings severely disrupts the acquisition of context conditioning while tone conditioning was minimally affected [22]. Several studies using the Morris water maze have reported ethanol-induced increase in latency to find the hidden platform [30,39]. But other studies in adult male animals with repeated ethanol treatments did not find impaired spatial learning and memory [1,8,18].

Ethanol sensitivity is known to vary with age [12, review 34]. Ethanol-induced disruption of long term potentiation (LTP) is more pronounced in hippocampal slices from periadolescent male rats than in adult male slices [26]. Following ethanol exposure, adolescent male rats show greater impairments in the acquisition of spatial learning and memory than adult rats [1,18, 33]. Although effects of ethanol have been studied in adolescent male animals, there is no study on ethanol's effects on the cognitive functioning in adolescent female animals. The present study was undertaken to investigate the effects of ethanol on the acquisition of spatial learning in adolescent female rats and compare them to those in adult females.

Materials and Methods

Animals and housing conditions

Twenty four adolescent and twenty four adult female Sprague-Dawley rats (Taconic, Germantown, MD) were housed in standard plastic cages, three to a cage, in a temperature and humidity controlled room within the animal facility. Food and water were available *ad*

libitum, and the colony was maintained on a 12-hr light–dark cycle with the lights on at 06:00 AM. Twenty four hours before behavioral testing, the back of the rat's head was dyed black for better contrast while tracking the animal in the water maze. All experimental protocols were approved by the institutional animal review committee, and the research was conducted in accordance with the requirements of NIH Guide for Care and Use of Laboratory Animals (1996).

Ethanol treatment

Age-dependent cognitive effects of ethanol were tested in prepubertal adolescent and adult female rats. The prepubertal adolescent rat was specifically chosen for the study to minimize the effects of gonadal hormones on ethanol-behavior interactions during adolescence. Rats were randomly assigned to one of two groups - saline or ethanol. Starting on postnatal day (PD) 30 for the adolescent group and PD 60 for the adult group, 30 min prior to behavioral testing, each rat received a single intraperitoneal (ip) injection of ethanol (2 g/kg); this dosage was based on earlier studies in adolescent and adult male rats showing age-dependent ethanol-induced cognitive impairments (18, 33). Animals received single daily ethanol injections for six days, five of which were on consecutive days. Controls rats received isovolumetric ip injections of saline on the same number of days.

Behavioral testing

Water maze apparatus—The Morris water maze (MWM) consisted of a circular pool (1.8 m diameter, 52 cm deep) whose interior was painted white. It was located in the center of a room dedicated to measuring this behavioral paradigm. The water temperature was carefully maintained between $25 \pm 2^\circ\text{C}$ with the help of a submersible digital water heating system (Cleveland Process Corporation, Homestead, FL), and the water was made opaque by adding non-toxic white paint. A removable 15 cm $\times$ 15 cm escape platform was introduced in the pool. The pool was divided into 4 quadrants and the escape platform was placed in one of the quadrants, one cm below the surface of the water. Performance was recorded and analyzed using a video tracking system (HVS Image, Hampton, UK). One day before water maze testing, all rats were habituated to the water, and taught to escape from the water by climbing onto the platform by placing their forepaws on the platform. Each rat was given four climb-on trials, and once on the platform it was allowed to sit there for 5 sec. The position of the platform used during habituation was not used again either for the cued visual task or during reference memory testing. A black curtain encircled the pool during the cued visual task. No data were collected and no drug administrations were made before, during or after habituation.

Experimental design—Behavioral testing protocols used were as described before [33]. An ethanol or saline injection was given thirty minutes before the first trial of the day. On PD 30 for adolescent rats and PD 60 for adult rats, each rat was tested in the cued visual task. This was followed by five days of testing in the hidden platform paradigm. On the fifth day of the hidden platform task, four hours after the last trial, each rat was subjected to a probe trial.

Visual cued task—Performance in the cued visual task was used to control for non-mnemonic sensorimotor functions such as swimming ability, motivation and visual function. Thirty min after ethanol or saline injection, each rat was put through the visible platform paradigm. This task required the rat to ignore the extra-maze information and use a black flag as a cue or beacon to indicate the location of the submerged escape platform; the escape platform was made “visible” by attaching a black flag to the platform. A black curtain encircled the pool preventing the animal from using extra-maze cues to find the platform. On each trial the subject was placed in the water, facing the edge of the tank in one of four pre-selected positions. The order of the start locations was varied in a quasi-random fashion such that in each block of four trials the subject started from each location one time and never started from

the same place on any four consecutive trials. For each batch of animals the platform remained fixed in one position (northwest, northeast, southwest and southeast) but was varied for different batches of animals. One test batch included 2–3 rats of each age and from each treatment group. There was a total 4–6 batches of rats. Rats were allowed to swim until they located the platform or 45 sec had elapsed. If the rat did not find the escape platform by the end of 45 sec, it was gently guided to the platform or was placed on the platform if it jumped off. The subject remained on the platform for 15 sec. There were four trials with an inter-trial interval of 60 sec. After the last trial, the rat was dried with paper towels and placed in a warm holding chamber before returning to the home cage.

Reference memory testing in the hidden platform paradigm—On five consecutive days, rats were injected with ethanol or saline, and tested in the water maze 30 min post injection. Trials were similar to those described for the visual cued task, except that the location of the escape platform was not visually marked, and the black curtain was removed so that the animal could see and use the visual clues provided on the walls of the room for spatial mapping (bright posters, large clock, wall phone, door etc). Rats were given four trials with an inter-trial interval of 60 sec. To rule out the possibility of the physical location of the platform as a confounding factor, the position of the platform was kept consistent within a batch of control and experimental rats but differed between different batches of animals. For each batch of animals, the location of the platform was different from that used in the cued visual task.

Probe trial—Four hours following the last reference memory trial, each rat was subjected to a probe trial. In the probe trial, the escape platform was removed from the pool and the rat was allowed to free swim for 45 sec. The purpose of the probe trial was to provide a method for evaluating the subject's knowledge of where the platform was located by quantifying the amount of time spent in the quadrant where the platform was previously situated [38] or visiting the location of the platform prior to its removal. During the probe trial the rat was released from a novel starting location (directly opposite to the platform location). No ethanol or saline was given prior to the probe trial. The time spent in the target quadrant (where the platform was located prior to its removal) was measured. A second more stringent measure referred to as visits to platform position ("pass through" or crossing over the platform location), was used to quantitate the probe trial in selected groups of animals. For additional controls, visits to four additional pre-selected areas identical to the platform location were also monitored. For both percent quadrant time and number of platform visits, higher numbers indicate better memory.

Data analysis

Repeated measures analysis of variance (RM ANOVA) using a mixed model approach (37) was used to examine the longitudinal and between-treatment group patterns, as well as the between-age group patterns separately for latency, distance and swim speed (SAS 9.2 for Windows; SAS Institute Inc., Cary, NC). Mixed model approach RM ANOVA was also used to analyze the probe trial quadrant data (% time spent in each quadrant). When there were no repeated measures, either a two-way ANOVA or an unpaired, two-tailed *t* test was used (Prism 3.00 for Windows; GraphPad Software Inc., San Diego CA). The level of significance was set at $p < 0.05$.

Blood ethanol levels

Separate groups of adolescent and adult female rats ($n = 12$ rats/age) were injected with 2 g/kg ethanol. Blood was collected by cutting 1–2 mm of the tail tip at 30, 60 or 240 min postinjection; each rat was bled only once. Blood was allowed to coagulate at room temperature for 30 min, and then centrifuged at $7,500 \times g$ for 3 min to obtain serum. Serum ethanol concentration was determined spectrophotometrically using the QuantiChrom™ Ethanol Assay Kit (BioAssay Systems, Hayward, CA). The assay is based on an improved dichromate method,

in which dichromate is reduced by ethanol to a bluish chromic (Cr^{3+}) product, and the intensity of the blue color is used as a direct measure of the ethanol concentration in the sample [13]. Serum was deproteinized by adding 10% TCA to the serum sample in a ratio of 2:1 (v/v). The serum-TCA mixture was centrifuged at $12,000 \times g$ for 5 min, and the supernatant was carefully transferred to a 96-well plate for assay. A working reagent (100 μl of Reagent A) was added to each well and the mixture was incubated for 8 min. This was followed by the addition of 100 μl of the Stop Reagent (Reagent B). Optical density was read in a spectrophotometer (BioWhittaker Microplate Reader 2001, BioWhittaker Inc., Walkersville, MD) at 610 nm. Serum ethanol concentration was calculated from the μg of ethanol in each sample, and expressed as mg/dl. A set of standards containing known amounts of ethanol were run in each assay.

Results

Blood ethanol level

The time courses of serum ethanol concentration were measured in adolescent and adult female rats at 30 min, 60 min and 240 min following a 2 g/kg dose of ethanol. A 2-way *age* $\times$ *time* ANOVA showed significant effects of both age ($F_{(1,12)} = 22.08$, $p < 0.0005$) and time of measure ($F_{(1,12)} = 23.09$, $p < 0.0004$). At 30 min postinjection there was no difference in serum ethanol levels between adolescent and adult female rats, but at 60 min adolescent females had significantly higher ethanol levels than adult female rats (Table I). In adolescent rats, 60 min after ethanol administration, blood ethanol levels were significantly higher than at 30 min. At 240 min following ethanol injection, blood ethanol levels were undetectable in both adolescent and adult rats.

Ethanol on cued visual task performance

To determine whether visual and motor coordination as well as motivation were compromised by ethanol treatments, rats were tested in the cued visual task. Thirty minutes after ethanol administration each rat was put through the visible platform task where the platform was marked with a black flag. Cued visual data (latency, distance and swim speed) were subjected to 2-way *age* $\times$ *treatment* ANOVA (Fig 1). There were significant effects of age on latency ($F_{(1,44)} = 9.858$, $p = 0.0030$), distance ($F_{(1,44)} = 5.432$, $p = 0.0244$) and swim speed ($F_{(1,44)} = 4.931$, $p = 0.0316$). There was no treatment effect on any of the test parameters (latency ($F_{(1,44)} = 0.03204$, $p = 0.8588$); distance ($F_{(1,44)} = 0.01094$, $p = 0.172$) or swim speed ($F_{(1,44)} = 0.8631$, $p = 0.3579$) between saline-treated and ethanol-treated rats.

Ethanol on the reference memory task performance

Adolescent and adult female rats were injected with a single dose of ethanol (2 g/kg) and tested 30 min later in the hidden platform water maze escape behavior on five consecutive days. Escape latency, distance, and swim speed data were subjected to RM ANOVA analyses using a mixed model approach for the longitudinal and between-drug interactions, as well as between age group patterns, separately for latency, distance and swim speed. The three-way interaction of *age* $\times$ *test day* $\times$ *treatment* was explored to determine if the difference between control and ethanol treated animals across the 5 days was different for adolescents and adult rats. The “within subjects” factor (the repeated factor) in these 3 models was test day, with age and treatment as the “between subjects” factors.

Swim speed—Mean swim speeds were recorded in adolescent and adult females every day after ethanol and saline treatment (Table II). The three-way interaction of *age* $\times$ *day* $\times$ *treatment* was not significant for swim speed. The *day* $\times$ *treatment* ($F_{(4,180)} = 2.80$, $p = 0.0272$) and *age* $\times$ *test day* ($F_{(4,180)} = 5.06$, $p = 0.0007$) interactions were significant. But *age* $\times$

treatment interaction was not significant ($F_{(1,44)} = 1.85, p = 0.1804$) indicating no ethanol effect on swim speed at either age.

Latency—The three-way interaction of *age* $\times$ *test day* $\times$ *treatment* was significant for latency ($F_{(4,172)} = 2.51, p = 0.0436$). Adolescent rats took significantly less time to find the hidden platform than adult rats ($F_{(4,172)} = 3.62, p = 0.0073$). Fig 2 shows the mean latency (collapsed across four trials) to reach the fixed platform by adolescent (Fig 2A) and adult (Fig 2B) saline-treated and ethanol-treated rats.

Adolescent ethanol on the latency to find the hidden platform—Repeated two-way ANOVA showed significant interactions between test day and treatment ($F_{(4,70)} = 3.227; p = 0.0173$) in the latency to find the hidden platform by saline-treated and ethanol-treated adolescent rats (Fig 2A). Latencies declined significantly over test days ($F_{(4,70)} = 12.01; p < 0.0001$). Compared to age-matched saline-treated controls, ethanol-treated rats took significantly longer to find the hidden platform ($F_{(1,70)} = 70.95, p < 0.0001$).

Adult ethanol on the latency to find the hidden platform—Repeated two-way ANOVA did not show significant interactions between test day and treatment ($F_{(4,70)} = 1.278; p = 0.2868$) in the latency to find the hidden platform by saline-treated and ethanol-treated adult rats (Fig 2B). Latencies declined significantly over test days ($F_{(4,70)} = 2.868; p < 0.0293$). Ethanol-treated rats took significantly longer to find the hidden platform compared to age-matched saline-treated controls ($F_{(1,70)} = 34.11, p < 0.0001$).

Distance—The three-way interaction of *age* $\times$ *test day* $\times$ *treatment* was not significant for distance, and was subsequently removed from the model. Fig 3 shows effects of ethanol on the swim distance in saline-treated and ethanol-treated adolescent (Fig 3A) and adult (Fig 3B) rats.

Adolescent ethanol on the distance traveled to find the hidden platform—Repeated two-way ANOVA showed significant interactions between test day and treatment ($F_{(4,70)} = 4.006; p = 0.0055$) in the distance traveled to find the hidden platform by saline-treated and ethanol-treated adolescent rats (Fig 3A). The distance traveled decreased significantly across test days ($F_{(4,70)} = 13.02; p < 0.0001$). Compared to age-matched saline-treated controls, ethanol-treated rats swam significantly greater distances to find the platform ($F_{(1,70)} = 60.97, p < 0.0001$).

Adult ethanol on the distance traveled to find the hidden platform—Repeated two-way ANOVA did not show any significant interaction between test day and treatment ($F_{(4,70)} = 1.278; p = 0.2868$) for the distance traveled to find the hidden platform by saline- and ethanol-treated adult rats (Fig 3B). Distance traversed decreased significantly over test days ($F_{(4,70)} = 9.723; p < 0.0001$). Ethanol-treated rats took significantly longer to find the hidden platform than age-matched saline controls ($F_{(1,70)} = 9.73, p = 0.0066$).

Ethanol on probe trial performance

A 45-sec probe trial was carried out after the hidden platform task with the platform removed from the maze (Fig 4). The percent time spent by adolescent (Fig 4A) and adult (Fig 4B) rats in each of the four quadrants were measured and statistically analyzed. Since the probe trial endpoints were percentages, the log transformation was used to better meet the assumptions of the ANOVA model with an unspecified covariance structure instead of an autoregressive covariance structure. The three-way interaction of *age* $\times$ *treatment* $\times$ *quadrant* was not significant. There was significant effect on *age* $\times$ *quadrant* ($F_{(3,36)} = 3.42, p < 0.0275$), but not for the *quadrant* $\times$ *treatment* ($F_{(3,36)} = 0.6225, p > 0.05$) or for the *age* $\times$ *treatment* ($F_{(1,36)} = 0.16, p > 0.05$).

In a subset of experimental and control animals, a second more stringent analysis of the probe trial data was performed, that of the number of visits (referred to as “pass through”) to the location of the platform prior to its removal (target location; Location TL) along with visits to four other equal-sized locations in the pool (Location 0, Location 1, Location 3, Location 4; Fig 5). Two-way ANOVA of *treatment* $\times$ *location* was carried out at each age separately. In adolescent rats (Fig 5A), the number of visits to the target platform location was significantly higher than to the other four locations ($F_{(4,40)} = 7.93$, $p < 0.0001$), and ethanol significantly reduced the number of visits to target location ($F_{(1,40)} = 4.965$, $p < 0.05$). Adult animals did not show any difference in the number of visits to the target location compared to visits to selected non-target locations ($F_{(4,50)} = 0.60$, $p > 0.05$). There was no treatment effect ($F_{(1,50)} = 0.4167$, $p > 0.05$).

Discussion

The main finding of the study is that ethanol disrupts spatial learning and memory in female adolescent rats. Adolescent female rats exposed daily to ethanol just before behavioral testing displayed marked impairments in learning in the simple fixed position hidden platform task in the Morris water maze, without affecting locomotor activity or performance in the cued visual task. In the probe trial, ethanol-exposed adolescent rats spent less time in the vicinity of the platform, before it was removed, than saline-treated adolescents. Using the same behavioral protocol as for adolescent rats, adult saline-treated animals did not learn the task as well as the younger rats. Ethanol-treated adult female rats took longer and swam greater distances to find the hidden platform compared to saline controls, and as with adolescent rats, ethanol in adult rats did not alter swim speed either in the cued visual task or in the hidden platform task. But in the probe trial, the ethanol-treated adults performed as well as the saline-treated adults both in the time spent in the target quadrant and in the number of visits made to the platform location. Thus in adolescent female rats ethanol affected latency, distance and number of visits to platform location in the probe trial, thereby specifically disrupting the acquisition of spatial learning. In adults, ethanol disturbed some of the test parameters (latency, distance) but not others (% target quadrant time or number visits to platform location in the probe trial).

Repeated ethanol treatment in adolescent female rats impaired the acquisition of place learning in the hidden platform task. This is further supported by data obtained from the probe trial testing. Ethanol did not alter any of the test parameters in the cued visual task – distance traveled or time taken to find the platform, indicating that impairments in the spatial reference memory were not due to compromised motor and/or visuoperceptive sensory functions, but due to proactive interference of spatial information processing in the performance of the task [2]. Previously, we and others have shown that ethanol causes deficits in the acquisition of spatial memory in adolescent male rats [1,18,33]. Together, these data suggest that repeated ethanol exposure attenuates acquisition of reference memory in adolescent rats of both sexes. These data differ from those reported by Hefner and Holmes [12]. They found normal sensitivity in the acquisition of Pavlovian fear conditioning in adolescent mice treated with ethanol. This difference could be due to the different behavioral paradigms used to measure cognitive functioning. Another possibility could be due to species differences in sensitivity to ethanol-induced cognitive impairments between adolescent rats and mice.

Data from the present study indicate that performance in the place version of the Morris water maze varies with age. Adolescent females perform significantly better than adults. This is the first report to indicate an age effect on spatial learning and memory in female rats. Since there was no *a priori* hypothesis that the acquisition of spatial memory would be affected by age, the treatment paradigm and testing models were identical at both ages. In the future, before testing for ethanol's behavioral effects in adult females, the spatial memory testing paradigm

needs to be refined by varying one or more parameters such as using longer search time, shorter inter-trial interval, increased number of trials per day or more number of test days,.

Adult ethanol-treated female rats showed significant differences in the classical Morris water maze parameters, that of time taken and distance traveled to reach the escape platform. But probe trial performances by ethanol-treated adult rats did not differ from that of adult saline-treated controls. Thus, in the adult female rat, ethanol's effects on the spatial memory appear complicated. These data are in line with earlier studies using adult male rats where ethanol did not disrupt memory with doses of up to 2 g/kg [1,18], but did in others [10,19,39]. Differences in findings between the present study and those reported by others [10,19,39] could rest upon several factors, particularly the behavioral task used to test for memory impairments. The radial arm maze and the T-maze paradigms were used in the reported studies [10,19,39], whereas the water maze was used in the present study. In the radial arm maze and the T-maze the animal has to make discreet choices within training trials but not so in the water maze where the animal has to find the correct platform location, and no such choices have to be made. Also, swimming is thought to be stressful in rats [23]. Whether ethanol impairs memory in female rats when tested in the T-maze or radial arm maze is not known.

One possibility for behavioral differences between adult and adolescent female rats may be due to ontogenic differences in ethanol clearance. Ethanol levels at the earliest time point, i.e. 30 min post injection did not differ between adolescent and adult rats. The levels peaked at 60 min in both adolescent and adult rats, with the adolescent rats having significantly higher levels than adult rats. By 4 hours blood ethanol levels at both ages were undetectable. These data differ from blood ethanol levels reported in an earlier study in adolescent and adult male rats [16]. Blood ethanol levels in both 30-days old and adult rats peaked at 30 min, but at all time points the levels were significantly higher in adult rats compared to adolescent rats. There appears to be gender differences in ethanol clearance in adolescent and adult rats. Since the blood ethanol levels at 60 min were significantly higher in adolescent females compared to adult rats, that elevated blood ethanol may have an effect on the hidden platform performance cannot be ruled out. Another possibility that needs to be considered is that over the course of the study, the adult rats may have developed greater tolerance (metabolic and/or pharmacodynamic) to the behaviorally disruptive effects of ethanol than adolescent animals. The visual task was carried out at the same time point following ethanol treatment as the reference task, and did not show any difference. The probe trials were done at 4 hours after the last trial of the hidden platform task and several hours after ethanol injection, i.e. when there was no detectable ethanol in blood, therefore, the impact of blood ethanol levels on probe trial performance appears to be minimal, if any at all. In the present study, blood ethanol levels were measured after a single acute injection. It is possible that repeated daily ethanol injections may have altered pharmacokinetics compared to following a single administration.

Alterations in anxiety-like behavior are known to modify learning and memory [31], and since ethanol has anxiolytic properties [15,27,40], one possibility for deficits in the Morris water maze performance in ethanol-treated rats could be alterations in their anxiety levels. If the animal is less fearful then it should spend less time in the vicinity of the pool wall and rapidly venture towards the center of the pool, thus taking less time and traveling a shorter distance to find the hidden platform. This did not appear to be the case. Ethanol-treated adolescent and adult rats took longer and swam greater distance to find the hidden platform. Reinforcing this finding, there was no correlation with any of the parameters in the visual cued task following ethanol exposure. Kameda *et al* [15] treated adult mice with ethanol and measured both anxiolytic and amnesic effects. They concluded that the anxiolytic effect could not account for the ethanol-induced memory deficits. Anxiety and thigmotaxis were not measured in the present study.

Intact adolescent and adult female rats were used in the present study. Effects of gonadal hormones and estrus cyclicity on ethanol-induced learning impairment in female rats were not investigated; adult female rats were not checked for estrous cyclicity. Since PD 30 female adolescent rats are prepubertal and do not have an open vagina [32] subjecting them to daily vaginal manipulation would have been traumatic and caused additional stress. Therefore, to keep the model consistent, adult females were also not subjected to any vaginal manipulation that would have been necessary to check for estrus cyclicity. How gonadal (estrogen, progesterone) and stress hormones modulate ethanol-induced cognitive functions in female rats needs careful investigation.

In conclusion, this is the first study to report cognitive functioning in adolescent female rats, and the effects of ethanol on it. Ethanol impaired the acquisition of spatial memory in adolescent female rats. In adult rats, ethanol affected some classical parameters of spatial learning but not others (probe trial performance). There was no effect of ethanol on the cued visual task performance at either age. Whether it is the relative lack of gonadal hormones, particularly estrogen, that underlies the ethanol-induced alterations in age-related learning differences, needs investigation. An interesting observation was made that adolescent female rats appear to have better spatial learning and memory than intact adult females. Future studies will look at the gender-specificity as well as the role of stress hormones in ethanol-induced deficits in cognitive function.

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Non-standard abbreviations

AUD	alcohol-use disorder
MWM	Morris Water Maze
PD	postnatal day

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Figure 1. Effect of ethanol on the visual cued task performance by adolescent and adult rats

Each rat received an injection of either saline or ethanol (2 g/kg, ip) 30 min prior to testing in the water maze. For details of the visual cued task see *Methods* section. Performance by adult rats significantly differed from adolescent rats. Compared to adolescent rats, adult rats took longer to reach the platform, swam greater distance and had slower swim speed. There was no difference between age-matched saline-treated and ethanol-treated rats. There was an *n* of 12 rats in each group.

Figure 2.

Effect of ethanol on escape latency to find the hidden platform on test days 1 through 5 of spatial reference memory task in adolescent ($n = 11$ saline-treated; $n = 12$ ethanol-treated; Fig 1A) and adult ($n = 12$ saline-treated; $n = 12$ ethanol-treated; Fig 1B) female rats. Each rat received a single daily injection of either saline or ethanol (2 g/kg, ip) 30 min prior to testing in the water maze. At both ages, the latency to find the hidden platform was significantly higher in ethanol exposed rats than saline-treated controls. Shown are the means $\pm$ sem of the average of each animal's four trials. ** $p < 0.01$, *** $p < 0.001$ compared to same-age, same-test day saline-treated controls.

Figure 3.

Effect of ethanol on the distance traveled to find the platform by (A) adolescent and (B) adult female rats. Rats received a single daily injection of ethanol (2 g/kg, ip) or saline 30 min prior to testing. Distance traveled by adolescent and adult ethanol-treated rats was significantly greater than same-age saline-treated controls. Saline treated adolescent rats swam shorter distance to the hidden platform than saline-treated adult rats. Values indicate mean $\pm$ sem of the average of each animals four trials. There were 12 rats in each group except the adolescent saline group which had 11 rats. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to same-age, same-test day saline-treated controls.

Figure 4.

Effect of ethanol exposure on the probe trial performance by (A) adolescent (B) adult female rats ($n = 11$ adolescent saline; $n = 12$ adolescent ethanol; $n = 12$ adult saline; $n = 12$ adult ethanol). The hidden platform was removed from the pool and the rat was allowed to search for the platform for 45 sec. The percent time spent by the rat in each of the four quadrants was measured. Both saline-treated and ethanol-treated adolescent rats spent significantly more time in the target quadrant (TQ) than in the other quadrants. Adult rats did not show preference for TQ. Values are mean $\pm$ sem. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to target quadrant of same-treatment adolescent rats

Fig 5.

Performance by ethanol- and saline-treated (A) adolescent and (B) adult female rats during the spatial probe trial was assessed by measuring pass throughs over specific locations in the pool during the 45 sec probe trial. Location 0 is the center of the pool, target location (TL) was the location of the platform prior to its removal, locations 1, 3 and 4 were virtual locations in each of the other quadrants. Each data point represents the number of visits $\pm$ sem. Saline-treated adolescent control rats crossed the target platform location more times than over the other locations, indicating their having learned the spatial location of the escape platform. Adult rats did not show any such preference. Ethanol-treated adolescent rats visited the target location less frequently than saline-treated adolescent females (** $p < 0.01$ compared to same-age other locations). No differences were observed in the adult females. ($n = 6$ rats in each treatment group and at each age).

TABLE I

Blood ethanol level in female rats

	30 min	60 min	240 min
adolescent	124.41 ± 14.96 (<i>n</i> = 4)	310.74 ± 39.14 ^{\$\$} (<i>n</i> = 4)	UN (<i>n</i> = 4)
adult	88.38 ± 15.58 (<i>n</i> = 4)	121.93 ± 13.81 ^{***} (<i>n</i> = 4)	UN (<i>n</i> = 4)

Values are mean ± sem (mg/dl).

UN = undetectable

^{\$\$} *p* < 0.005 compared to 30 min adolescent females

^{***} *p* < 0.001 compared to same-time adolescent females

TABLE II

Mean swim speed (cm/min) in the hidden platform task

	Day 1	Day 2	Day 3	Day 4	Day 5
<i>adolescent</i>					
saline (<i>n</i> = 11)	24.12 ± 0.97	22.98 ± 1.35	21.41 ± 1.49	22.85 ± 1.31	21.29 ± 0.90
ethanol (<i>n</i> = 12)	20.80 ± 1.23	19.14 ± 1.01	20.48 ± 1.34	21.90 ± 1.09	21.16 ± 1.50
<i>adult</i>					
saline (<i>n</i> = 12)	25.99 ± 0.92	19.77 ± 1.21	19.16 ± 1.82	18.46 ± 1.37	18.12 ± 1.74
ethanol (<i>n</i> = 12)	19.03 ± 1.52	12.38 ± 1.58	16.84 ± 1.30	15.79 ± 0.84	15.95 ± 1.49

Values are means from 4 trials ± sem. There was no significant difference between same-age, same-day saline- and ethanol-treated rats