Pharmacology, Biochemistry and Behavior 96 (2010) 476–487

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Repeated binge ethanol administration during adolescence enhances voluntary sweetened ethanol intake in young adulthood in male and female rats

Antoniette M. Maldonado-Devincci [a], Kent K. Alipour [a], Laura A. Michael [a], Cheryl L. Kirstein [a,b,*]

[a] Department of Psychology, Cognitive and Neurosciences, University of South Florida 4202 E. Fowler Avenue Tampa, FL 33620, United States
[b] Department of Physiology and Molecular Pharmacology, University of South Florida College of Medicine, Tampa, FL 33612, United States

ARTICLE INFO

Article history:
Received 4 June 2009
Received in revised form 7 July 2010
Accepted 10 July 2010
Available online 15 July 2010

Keywords:
Alcohol
Repeated binge administration
Dose–response
Sex differences
Adolescence

ABSTRACT

Binge alcohol consumption is a rising concern in the United States, especially among adolescents. During this developmental period alcohol use is usually initiated and has been shown to cause detrimental effects on brain structure and function as well as cognitive/behavioral impairments in rats. Binge models, where animals are repeatedly administered high doses of ethanol typically over a period of three or four days cause these effects. There has been little work conducted aimed at investigating the long-term behavioral consequences of repeated binge administration during adolescence on later ethanol-induced behavior in young adulthood and adulthood. The repeated four-day binge model may serve as a good approximate for patterns of human adolescent alcohol consumption as this is similar to a "bender" in human alcoholics. The present set of experiments examined the dose–response and sex-related differences induced by repeated binge ethanol administration during adolescence on sweetened ethanol (Experiment 1) or saccharin (Experiment 2) intake in young adulthood. In both experiments, on postnatal days (PND) 28–31, PND 35–38 and PND 42–45, ethanol (1.5, 3.0 or 5.0 g/kg) or water was administered intragastrically to adolescent rats. Rats underwent abstinence from PND 46–59. Subsequently, in young adulthood, ethanol and saccharin intake were assessed. Exposure to any dose of ethanol during adolescence significantly enhanced ethanol intake in adulthood. However, while female rats had higher overall g/kg intake, males appear to be more vulnerable to the impact of adolescent ethanol exposure on subsequently increased ethanol intake in young adulthood. Exposure to ethanol during adolescence did not alter saccharin consumption in young adulthood in male or female rats. Considering that adolescence is the developmental period in which ethanol experimentation and consumption is usually initiated, the present set of experiments demonstrate the importance of elucidating the impact of early binge-pattern ethanol exposure on the subsequent predisposition to drink later in life.

Published by Elsevier Inc.

1. Introduction

Recent evidence has shown a high rate of experimentation with alcohol during adolescence. According to the 2009 Monitoring the Future study, 15% of 8th graders, 30% of 10th graders, and 44% of 12th graders reported current use of alcohol, defined as consumption of at least one alcoholic beverage in the past 30 days, and 5% of 8th graders, 16% of 10th graders, and 27% of 12th graders reported being drunk in the last 30 days (Johnston et al., 2009). Binge drinking, often defined as the consumption of five or more drinks for men or four or more drinks for women on a single occasion (Wechsler et al., 1994), has been labeled the number one source of preventable morbidity and mortality for more than 6 million college students in the United States (Wechsler et al., 1995). Furthermore, recent longitudinal data indicate about one half of males and one-third of females that engaged in binge drinking during adolescence continued to engage in similar binge drinking patterns in adulthood (McCarty et al., 2004). These statistics are of concern, as evidence supports the notion that early exposure to alcohol may be a significant predictor of later alcohol consumption, dependence, and various psychiatric disorders (Grant et al., 2001; Hasin and Glick, 1992; Robin et al., 1998).

Sex differences have been observed in alcohol consumption (Grant and Harford, 1991; de Lint and Schmidt, 1971; Wilsnack and Wilsnack, 1995). Men are more likely to drink than women, and drink in larger amounts (Wilsnack et al., 2000). However, in the United States, the number of cases of alcohol intoxication among younger women is rising (Wilsnack and Wilsnack, 1995), and a dramatic increase in the estimated prevalence of alcoholism among women living in North America over the last three decades has been reported (de Lint and Schmidt, 1971; Grant and Harford, 1991). Furthermore, women have been found to be especially vulnerable to the medical risks of heavy drinking, including liver diseases and psychiatric problems (Bradley et al., 1998). Together, these data not only highlight the changing trend in ethanol (EtOH)

* Corresponding author. Psychology Department-PCD 4118G, University of South Florida, 4202 East Fowler Avenue, Tampa, FL 33620, United States. Tel.: +1 813 974 9626; fax: +1 813 974 4617.
E-mail address: kirstein@cas.usf.edu (C.L. Kirstein).

0091-3057/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.pbb.2010.07.008

consumption among females, but also emphasize the possible implications of heavy drinking among women.

The behavioral effects of alcohol exposure during adolescence and the sex differences that may mediate long-term behavioral outcomes cannot be systematically examined in humans given it is unethical to administer EtOH to human adolescents; therefore, animal models have been developed to investigate these effects. In these models, sex differences have been observed in both alcohol consumption and preference. Females have been reported to consume more EtOH than males in both rat and mouse models (Almeida et al., 1998; Blanchard et al., 1993; Eriksson and Pikkarainen, 1968; Hutchins et al., 1981; Juarez and Barrios de Tomasi, 1999; Lancaster et al., 1996; Lancaster and Spiegel, 1992; Li and Lumeng, 1984), and maintain a preference for EtOH for a longer duration (Almeida et al., 1998).

Research has shown that EtOH exposure during adolescence may result in negative consequences such as impaired spatial learning (Markwiese et al., 1998) and intermittent EtOH (3.0 g/kg for two consecutive days at 48 h intervals) induced chronic neurobehavioral deficiencies (Pascual et al., 2007). Lower doses of daily EtOH (0.5 g/kg) may not induce these same effects (Acheson et al., 2001). Moreover, recent work indicates that binge four-day EtOH administration has greater detrimental effects on brain structure and function in adolescent as compared with adult rats (Crews et al., 2000; Monti et al., 2005). EtOH exposure during adolescence has also been reported to cause dose-dependent cognitive and behavioral impairments (Crews et al., 2006), however chronic intermittent EtOH exposure during adolescence does not induce similar effects (Silvers et al., 2003, 2006). Taken together, these data indicate normal adolescent brain and behavioral development may be altered by the deleterious effects of repeated binge EtOH exposure.

The present experiment aimed to investigate the long-term dose-dependent behavioral consequences of repeated binge EtOH administration during adolescence on voluntary sweetened EtOH (Experiment 1) or voluntary saccharin (Experiment 2) intake in young adulthood in male and female rats. The four-day repeated binge model was chosen given that in humans frequent binge drinkers in college report more than three binge drinking episodes per week, and those that binge drank in college reported similar rates of binge drinking in high school (Wechsler et al., 1994, 1995). Given that human adolescents prefer low EtOH, high carbohydrate alcoholic beverages such as beer, wine coolers, and various sweetened drinks (Substance Abuse and Mental Health Services Administration, 2001; Wechsler et al., 2000), saccharin was expected to elevate voluntary sweetened EtOH consumption in the 30-min the animals were given free access to consume this solution in Experiment 1. To ensure the elevated sweetened EtOH intake observed in young adulthood following adolescent EtOH exposure was not merely attributed to the sweetener used, voluntary saccharin intake data were collected in Experiment 2. Based on previous reports (Crews et al., 2006; Matthews et al., 2002), long-term dose-dependent changes in drinking behavior were expected in young adulthood following adolescent EtOH exposure. Additionally, female rats were expected to exhibit greater voluntary EtOH intake as compared to males, given that female rodents generally consume more EtOH than males (Almeida et al., 1998; Blanchard et al., 1993; Eriksson and Pikkarainen, 1968; Hutchins et al., 1981; Juarez and Barrios de Tomasi, 1999; Lancaster et al., 1996; Lancaster and Spiegel, 1992; Li and Lumeng, 1984).

2. Methods

2.1. Subjects

One hundred and thirty six male (n = 70) and female (n = 66) Sprague-Dawley rats were used for Experiment 1, 43 male (n = 22) and female (n = 21) rats were used for Experiment 2, 20 female rats were used for Experiment 3 (Harlan Laboratories, Indianapolis, IN), all of which were derived from established breeding pairs at the University of South Florida, Tampa and used as subjects in the present set of experiments. Litters were sexed and culled to 10 pups per litter on postnatal day (PND) 1, with the day of birth designated as PND 0. Pups remained with their respective dams until PND 21, when pups were group-housed with same-sex littermates in groups of two or three. Animals were maintained on a 12:12 h light: dark cycle (lights on at 0700 h), in a temperature and humidity-controlled vivarium. Animals were allowed free access to food and water throughout the experiment. No more than one male or female pup per litter was used in any given condition. Animals were randomly assigned to conditions of 1.5, 3.0 or 5.0 g/kg EtOH (25% v/v in water; Pharmaco-Aaper, Shelbyville, KY) or an isovolumetric administration of water (Hunt et al., 2000; Nixon and Crews, 2002). For experiment 1, control groups contained 12 animals per group, and EtOH-treated animals contained 13 per group, 13 animals were lost due to improper intubation. For experiment 2, all groups contained 12 animals per group and 5 animals were lost due to improper intubation. For experiment 3, all groups contained 10 animals per group. Maintenance and treatment of the animals were within the guidelines for animal care by the National Institutes of Health (Public Health Service Policy on Humane Care and Use of Laboratory Animals, NIH, 2002). Animals were intragastrically administered EtOH or water beginning on PND 28 (described below), and assessment of sweetened voluntary EtOH intake (Experiment 1) or saccharin intake (Experiment 2) began on PND 60 to PND 69, (i.e., around the onset of young adulthood) or sweetened voluntary EtOH intake (Experiment 3) from PND 72 to PND 81 (described below).

2.2. Apparatus

Male and female rats were intragastrically administered water or 25% v/v EtOH in water via daily intubation using a 12-cm length of polyethylene tubing (PE-50; Becton Dickinson and Company; Sparks, MD) attached to a 21.5 gauge needle and a disposable syringe (Hunt et al., 2001). The intubation volume was different depending on the dose of EtOH administered, as all EtOH doses were administered on a gram of EtOH per kilogram of body weight basis. Assessment for voluntary intake of sweetened EtOH, water and saccharin was performed with 500 mL glass bottles with double-ball bearing tips (Ancare Corporation, Bellmore, NY). Previous work has indicated voluntary EtOH intake is higher in adolescent rats when using double-ball bearing tips as compared to standard open-ended tips (Doremus et al., 2005).

2.3. Procedure

The present experiment was conducted in three phases over a period of 41 days. The first phase was repeated binge EtOH or water pretreatment during adolescence beginning on PND 28 and continuing through PND 45. The second phase was abstinence, which began on PND 46 and continued through PND 59. The final phase began in young adulthood and assessed sweetened voluntary EtOH intake (Experiment 1) or voluntary saccharin intake (Experiment 2), initiated on PND 60 and concluded on PND 69. Experiment 3 assessed voluntary EtOH intake in adult females only beginning on PND 72 and ended on PND 81.

2.4. Experiment 1: repeated binge ethanol pretreatment

On PND 28–31, PND 35–38, and PND 42–45, animals were intragastrically administered EtOH (25% v/v EtOH diluted from 95% EtOH in water) or water (n = 67) using one of three doses (1.5, n = 23; 3.0, n = 22; or 5.0, n = 24 g/kg/ig). Animals were administered either EtOH or an equivalent isovolumetric administration of water.

Therefore, there was a control group for each dose (1.5, n = 23; 3.0 n = 23; 5.0 n = 20), with one group administered EtOH and the control group administered water equivalent in volume to that of the EtOH group. Using a repeated four-day binge administration, adolescent rats were transported to the laboratory, weighed, and administered their respective EtOH dose or water. This procedure was repeated every 24 h on treatment days between 0900–1200 h during the light cycle. On PND 32–34 and PND 38–41, animals were left undisturbed in the colony room, except for regular cage maintenance.

2.5. Experiments 2 and 3: repeated binge ethanol pretreatment

Given that the same general pattern of sweetened EtOH consumption was observed in young adulthood in rats pretreated with EtOH during adolescence in Experiment 1, the highest pretreatment dose (5.0 g/kg/ig) was chosen for Experiments 2 and 3. Similar to Experiment 1, on PND 28–31, PND 35–38, and PND 42–45, animals were intragastrically administered EtOH (25% v/v EtOH diluted from 95% EtOH in water; 5.0 g/kg/ig) or an equivalent isovolumetric administration of water. This procedure was repeated every 24 h on treatment days between 0900–1200 h during the light cycle. On PND 32–34 and PND 38–41, animals were left undisturbed in the colony room, except for regular cage maintenance.

2.6. Abstinence

For Experiments 1, 2 and 3, beginning on PND 46 until PND 59, all rats underwent abstinence. During this time, animals were left undisturbed in the colony room until young adulthood, except for regular cage maintenance.

2.7. Experiment 1: adulthood voluntary sweetened ethanol intake

Beginning on PND 60 through PND 69, young adult rats were assessed for voluntary sweetened EtOH intake using a limited access two-bottle choice paradigm. Fresh bottles were presented to all animals daily with one bottle containing a saccharin/EtOH solution and the other bottle containing tap water. The saccharin/EtOH solution was composed of 0.5% saccharin/10% EtOH. Saccharin (Alta Aesar, Ward Hill, MA) was presented as weight/volume and EtOH was presented as volume/volume. The saccharin/EtOH concentration was selected based on optimal voluntary intake in pilot data. The side of presentation of the saccharin/EtOH and water bottle was alternated daily to avoid development of a side preference. Bottles were weighed to the nearest 0.1 g before and after the 30-min access period. The difference in weight indicated the amount of EtOH consumed, and data were presented as grams of EtOH per kilogram of body weight (g/kg) for the 30-min session. Spillage was accounted for by placing saccharin/EtOH and water bottles in a similar holding cage unoccupied by a rat. The difference calculated between the presentation and removal of the bottle from the holding cage accounted for spillage and was subtracted from the daily difference calculated for each rat.

On each day, beginning on PND 60 through PND 69, animals were transported to the laboratory and weighed. With free access to food and water, animals were placed into a holding cage for 30-min to allow them to acclimate to the behavioral testing room. After a timed 30-min interval, the original water bottle was removed, and rats were simultaneously presented with the saccharin/EtOH bottle and a second bottle containing tap water. The bottles were previously weighed to the nearest 0.1g (as indicated above), and were available to the animal for 30-min. After the 30-min access period, both bottles were removed and again weighed to the nearest 0.1 g. All animals were presented with the original water bottle and remained in the behavioral testing room for an additional 60-min and then all rats were returned to the colony room. This procedure was repeated each day between 0900–1200 h during the light cycle.

2.8. Experiment 2: adulthood voluntary saccharin intake

Given a sweetened EtOH solution was used in Experiment 1, for Experiment 2 voluntary saccharin intake was assessed in young adulthood. Experiment 2 was conducted to assess if the enhanced sweetened EtOH observed in Experiment 1 may be attributed to the EtOH in the solution as opposed to simple enhanced consumption of a palatable sweetened saccharin solution (Experiment 2). All procedures were similar to Experiment 1, except all animals were given a choice between a saccharin solution (0.5% w/v) and water for 30-min using a limited-access two-bottle choice paradigm.

2.9. Experiment 3: lavage and adulthood voluntary sweetened ethanol intake

Beginning on PND 60 through PND 71, female rats underwent daily vaginal lavage. Each female rat was individually removed from the holding cage and had a plastic 200 μL pipette tip containing 10 μL of sterile room temperature saline (0.9% NaCl) inserted approximately 1 cm into the vagina. The saline was deposited into the vagina and immediately following the vaginal fluid was collected and placed on a clean glass slide. A different glass slide and a clean pipette tip was used for each rat. Immediately following the collection the rat was returned to the homecage and returned to the colony room. This procedure was performed every day.

Beginning on PND 72 through PND 81, adult female rats were assessed for voluntary sweetened EtOH intake. All procedures were similar to Experiment 1. From PND 72–81, animals continued to undergo daily vaginal lavage immediately following the EtOH intake session (described below).

2.10. Design and analyses

2.10.1. Experiment 1

Voluntary sweetened EtOH intake did not significantly differ between any of the control groups within sex; therefore, data were collapsed across all control groups for each sex. To conduct a detailed analysis of the progression of increased voluntary sweetened EtOH intake observed in young adulthood in male and female rats, voluntary sweetened EtOH intake data were analyzed using a three-factor mixed model design ANOVA with Sex (2; Male or Female) and Dose (4; 0.0, 1.5, 3.0 or 5.0 g/kg/ig) as between-subjects factors and Days (5; PND 60–61, PND 62–63, PND 64–65, PND 66–67 and PND 68–69) as a repeated measure. Preference for sweetened EtOH intake data were analyzed using a three-factor mixed model ANOVA with Sex (2; Male or Female) and Dose (4; 0.0, 1.5, 3.0 or 5.0 g/kg/ig) as between-subjects factors and Days (5; PND 60–61, PND 62–63, PND 64–65, PND 66–67 and PND 68–69) as a repeated measure. Given sex differences were observed in control animals for voluntary sweetened EtOH intake, data were equated and analyzed as a percent of control to identify if females were indeed at increased vulnerability for enhanced voluntary sweetened EtOH intake in young adulthood relative to males, therefore, data were analyzed with a three-factor mixed model ANOVA with Sex (2; Male or Female) and Dose (3; 1.5, 3.0 or 5.0 g/kg/ig) as between-subjects factors and Days as a repeated measure. Subsequent post-hoc tests were used to isolate effects in the presence of an interaction (Newman–Keuls, Tukey's Multiple Comparison Test, and simple-effects). The level of significance was set at 0.05 (SuperAnova, Abacus Concepts, Berkeley, CA).

2.10.2. Experiment 2

Voluntary saccharin consumption was analyzed using a three-factor mixed model design ANOVA with Sex (2; Male or Female) and Dose

(2; 0.0 or 5.0 g/kg) as between-subjects factors and Days (5; PND 60–61, PND 62–63, PND 64–65, PND 66–67 and PND 68–69) as a repeated measure. Subsequent post-hoc tests were used to isolate effects in the presence of an interaction (Newman–Keuls, Tukey's Multiple Comparison Test, and simple-effects). The level of significance was set at 0.05.

2.10.3. Experiment 3

Voluntary EtOH consumption was analyzed using a two-factor design ANOVA with Dose (2; 0.0 or 5.0 g/kg) as between-subjects factors and Estrous Stage nested in Dose (4; Proestrus, Estrus, Metestrus and Diestrus). Subsequent post-hoc tests were used to isolate effects in the presence of an interaction (Newman–Keuls). The level of significance was set at 0.05.

3. Results

3.1. Experiment 1

3.1.1. Weight

To examine changes in weight gain during adolescence and adulthood in male and female rats treated with EtOH relative to water, data were analyzed separately for each sex using a two-way analysis of variance for Days and Dose during adolescence and adulthood (data are depicted in top panel in Table 1). For males, a significant main effect of Days [$F(33, 726) = 7413.82$, $p<0.0005$] and a significant two-way interaction of Dose by Days was found during adolescence (PND 28–31, PND 35–38 and PND 42–45; [$F(33, 726) = 2.41$, $p<0.05$]). Post-hoc analyses indicate there were no significant differences in weight on any given day during EtOH pretreatment during adolescence. Control rats showed significant increases in weight gain across all days [$F(11, 371) = 227.6$, $p<0.0005$], however rats treated with 1.5 [$F(11, 155) = 79.8$, $p<0.0005$] or 3.0 [$F(11, 143) = 103.3$, $p<0.0005$) g/kg EtOH showed significant increases in weight from PND 35–38 and PND 42–45, and rats treated with 5.0 g/kg EtOH showed significant increases in weight on PND 29, PND 35–38 and PND 42–45 [$F(11, 143) = 91.06$, $p<0.0005$]. A significant main effect of Days was also found [$F(11, 726) = 7413.82$, $p<0.0005$], but not a significant main effect of Dose ($p>0.05$). For females, during adolescent pretreatment, the two-way interaction of Dose by Days failed to reach statistical significance ($p>0.05$). However, a significant main effect of Days [$F(11, 682) = 6808.94$, $p<0.0005$] and Dose [$F(3, 62) = 3.40$, $p<0.05$] were found. Posthoc analyses indicate female rats treated with the 5.0 g/kg dose of EtOH during adolescence weighed less than controls rats.

In young adult (PND 60–69) males, a significant two-way interaction of Dose by Days [$F(27, 594) = 2.01$, $p<0.05$] and a significant main effect of Days [$F(9, 594) = 1021.12$, $p<0.0005$] were found. The main effect of Dose failed to reach statistical significance ($p>0.05$). Posthoc analyses indicate there were no significant differences in weight between any of the groups on any given day during the voluntary sweetened EtOH intake during young adulthood. Control male rats showed a significant increase in weight gain across all days during young adulthood [$F(9, 329) = 597.1$, $p<0.0005$], however rats treated with 1.5 [$F(9, 129) = 504.7$, $p<0.0005$] and 5.0 [$F(9, 119) = 374.9$, $p<0.0005$] g/kg EtOH during adolescence showed sporadic increases in weight across days in young adulthood, while rats treated with 3.0 g/kg EtOH during adolescence only showed a significant increase in weight from PND 61–62 [$F(9, 119) = 488.6$, $p<0.0005$]. In young adult females, the two-way interaction of Dose by Days ($p>0.05$) and the main effect of Dose ($p>0.05$) failed to reach statistical significance. However, a significant main effect of Days [$F(9, 558) = 302.58$, $p<0.0005$] was revealed.

3.2. Voluntary ethanol intake

To conduct a detailed analysis of the impact of sex and EtOH or water dose in adolescence on voluntary sweetened EtOH intake in young adulthood, data were analyzed with Days as a repeated measure. Overall, a significant three-way interaction of Sex by Dose by Days was found [$F(12, 512) = 2.71$, $p<0.003$]. Additionally, significant two-way interactions of Sex by Day [$F(4, 512) = 5.73$, $p<0.0005$] and Dose by Days were found [$F(12, 512) = 5.94$, $p<0.0005$]. Significant main effects of Sex [$F(1, 128) = 299.66$, $p<0.0005$], Dose [$F(3, 128) = 241.70$, $p<0.001$] and Days [$F(4, 512) = 200.07$, $p<0.0005$] were also found. As depicted in both panels of Fig. 1, voluntary sweetened EtOH consumption increased across days in young adulthood in both males [$F(4, 264) = 100.26$, $p<0.0005$] and females [$F(4, 248) = 101.05$, $p<0.0005$]. Fig. 1A illustrates male rats administered any dose of EtOH during adolescence consumed significantly more sweetened EtOH relative to controls at all time points {Dose by Days [$F(12, 264) = 8.78$, $p<0.0005$] and Dose [$F(3, 66) = 131.95$, $p<0.0005$]}. Additionally, males rats administered 5.0 g/kg EtOH ($\text{mean} \pm \text{SEM} = 1.02 \pm 0.09$) during adolescence consumed significantly more sweetened EtOH relative to those administered 1.5 ($\text{mean} \pm \text{SEM} = 0.84 \pm 0.07$) and 3.0 ($\text{mean} \pm \text{SEM} = 0.81 \pm 0.06$) g/kg EtOH during adolescence on PND 64–65 {Dose [$F(3, 66) = 24.75$, $p<0.0005$]}. On PND 66–67 {Dose [$F(3, 66) = 41.50$, $p<0.0005$]}, and PND 68–69 {Dose [$F(3, 66) = 70.06$, $p<0.0005$]}, male rats administered 5.0 g/kg EtOH during adolescence consumed significantly more sweetened EtOH relative to those administered 3.0 g/kg EtOH (Fig. 1A). Water-treated males achieved stable voluntary sweetened EtOH intake in young adulthood as indicated by a significant increase in voluntary sweetened EtOH consumption from PND 60–61 ($\text{mean} \pm \text{SEM} = 0.20 \pm 0.02$) to PND 62–63 ($\text{mean} \pm \text{SEM} = 0.42 \pm 0.03$), with no future significant differences in sweetened EtOH consumption from PND 62–63 through PND 68–69 ($\text{mean} \pm \text{SEM} = 0.49 \pm 0.04$) {Days [$F(4, 128) = 15.03$, $p<0.0005$]}.

Fig. 1B illustrates increases in voluntary sweetened EtOH intake across days in water-treated {Days: 0.0 [$F(4, 132) = 57.28$, $p<0.0005$]} and EtOH-treated {Days: 1.5 [$F(4, 36) = 17.51$, $p<0.0005$], 3.0 [$F(4, 36) = 24.69$, $p<0.0005$], and 5.0 [$F(4, 44) = 21.99$, $p<0.0005$]} female rats. Female rats administered any dose of EtOH during adolescence consumed significantly more EtOH relative to controls on all days {Dose [$F(3, 61) = 101.05$, $p<0.001$]; Days [$F(4, 248) = 101.05$, $p<0.0005$] (Fig. 1B)}. Post-hoc analyses for the significant main effect of Dose indicate female rats administered 5.0 g/kg EtOH during adolescence consumed significantly more sweetened EtOH in young adulthood relative to those administered 1.5 ($p<0.005$) and 3.0 ($p<0.02$) g/kg EtOH during adolescence. At all doses and time points, females consumed significantly more EtOH than males {0.0: [Days by Sex $F(4, 260) = 18.57$, $p<0.0005$]; 1.5 [Sex: $F(1, 21) = 42.57$, $p<0.0005$; Days: $F(4, 84) = 42.83$, $p<0.0005$]; 3.0 [Sex: $F(1, 20) = 54.53$, $p<0.0005$; Days: $F(4, 80) = 45.34$, $p<0.0005$]; 5.0 [Sex: $F(1, 22) = 49.28$, $p<0.0005$; Days: $F(4, 88) = 42.55$, $p<0.0005$].

To ensure the patterns of greater sweetened EtOH intake in EtOH-pretreated rats observed in Fig. 1 were not due to alterations in general consumption patterns (i.e., greater EtOH consumption accompanied by greater water intake), data were analyzed as preference ratios for EtOH {(EtOH (ml)/[EtOH (ml) + Water (ml))*100]}. A significant main effect of Dose [$F(3, 128) = 71.05$, $p<0.0005$] and Days [$F(4, 512) = 51.27$, $p<0.0005$] were found. Given there were no significant main effects or interactions that included the Sex factor and there was a trend for a Dose by Days effect [$F(12, 512) = 1.78$, $p = 0.058$], data were analyzed separately for each sex using a two-way ANOVA of Days by Dose. As shown in Fig. 2A for males, a significant main effect of Dose [$F(3, 66) = 51.80$, $p<0.0005$] was found with rats exposed to any dose of EtOH during adolescence exhibiting a significant increase in preference for EtOH relative to those administered water during

Table 1

Experiment 1	Male 0.0		Male 1.5		Male 3.0		Male 5.0		Female 0.0		Female 1.5		Female 3.0		Female 5.0	
28	97.9		96.6		97.6		95.4		88.5		82.5		84.1		80.2	
29	104	*	100.9		102.5		100.8		93.6	*	86.3		87.8		83.5	
30	110.7	*	106.3		108.5		106.6	*	98.5	*	91.5		93		88.7	*
31	118.2	*	113.2	*	115.5	*	113.1	*	103.6	*	96	*	98.4	*	93.3	*
35	148.3	*	142.7	*	146.2	*	143.2	*	125.9	*	118	*	119.1	*	114.5	*
36	154.9	*	149.4	*	152.5	*	149.1	*	130.8	*	123.3	*	124.1	*	119.6	*
37	163	*	156.2	*	159.9	*	156.5	*	135.8	*	128.6	*	129.3	*	124.8	*
38	169.7	*	163.3	*	166.5	*	163.3	*	140.1	*	133.8	*	134.1	*	129.4	*
42	202.8	*	193.8	*	196.5	*	194.2	*	156.9	*	151.8	*	151.9	*	149.7	*
43	208	*	199.6	*	202.6	*	200.4	*	160.6	*	155	*	153.8	*	151.1	*
44	215.3	*	206.8	*	209.3	*	206.2	*	164.7	*	159.1	*	158.2	*	154.9	*
45	223.5	*	213.4	*	215.3	*	211.2	*	168.9	*	164	*	162.7	*	158.8	*
60	324.3		316.8		317.6		318.1		211.3		208		202.2		200.9	
61	327.4	*	320.6		320.1		319.4		213.8	*	209.3		203.7		202.3	
62	332.4	*	324.6		325.1	*	325.4	*	216		211		205.7		204.2	
63	335.8	*	330	*	329.7		329.4	*	217.2		212.2		206.8		207	
64	341.7	*	334.5	*	333.1		334.1	*	220.1	*	214.7		209.1		208.5	
65	346.3	*	338.3		336.2		337.5		222.3		217.4		210.8		211.1	
66	351.3	*	342.9	*	340.3		342.8	*	225.1	*	220.1		214.6	*	214.2	
67	356.3	*	346.6		345.1		348.4	*	227.7	*	222.7		217.4		216.2	
68	360.7	*	350.9	*	349.3		351.5		230.6	*	224.9		219.6		218.8	
69	364	*	353.7		351.5		354.2		233.3	*	228.8	*	221.8		221.9	

Experiment 2	Male 0.0		Male 5.0		Female 0.0		Female 5.0	
28	97.9		95.4		83.2		82.9	
29	104		100.8		88.5	*	85.9	
30	110.7		106.6		92.3	*	89.3	
31	118.2	*	113.1		97.2		93.3	
35	148.3	*	143.2	*	119.2	*	115	*
36	154.9	*	149.1		123.6	*	118.8	
37	163	*	156.5	*	1282	*	121.6	
38	169.7	*	163.3	*	132.9	*	126.4	
42	202.8	*	194.2	*	149.6	*	145.6	*
43	208		200.4		151	*	148.9	
44	215.3	*	206.2		155.2	*	152	
45	223.5		211.2		158.2		155.6	
60	324.3		303.3		196.7		197.6	
61	327.4		306.9		195.9		196.3	
62	332.4		311.9	*	198.3		197.6	
63	335.8		317.2	*	199.8		200.7	
64	341.7		321.7	*	203.3	*	205.4	*
65	346.3		324		205.1		206.1	
66	351.3	*	329.4	*	207.7		206.1	
67	356.3		333.8	*	209.9		209.1	
68	360.7		339.4	*	210.7		212.7	
69	364		342.7		213.2		215.3	

Experiment 3	Female 0.0		Female 5.0	
28	82		83.8	
29	86		86.2	
30	90.1		89.5	
31	94.9	*	93.8	
35	117.3	*	115.6	*
36	121.6	*	119.3	
37	126.8	*	124.3	
38	131.3	*	128.3	
42	149	*	148.6	*
43	152.1		151.9	
44	155.9		156.2	
45	158.9		159.7	
72	224.8		228.1	
73	226.2		228.3	
74	226.8		230.5	
75	228.9		232.7	
73	229.9		23.1	
77	232.2		235.8	
78	232.8		237.6	
79	233.8		237.2	
80	233.2		238.4	
81	233.4		239.5	

* Indicates significant increase in weight from previous day.

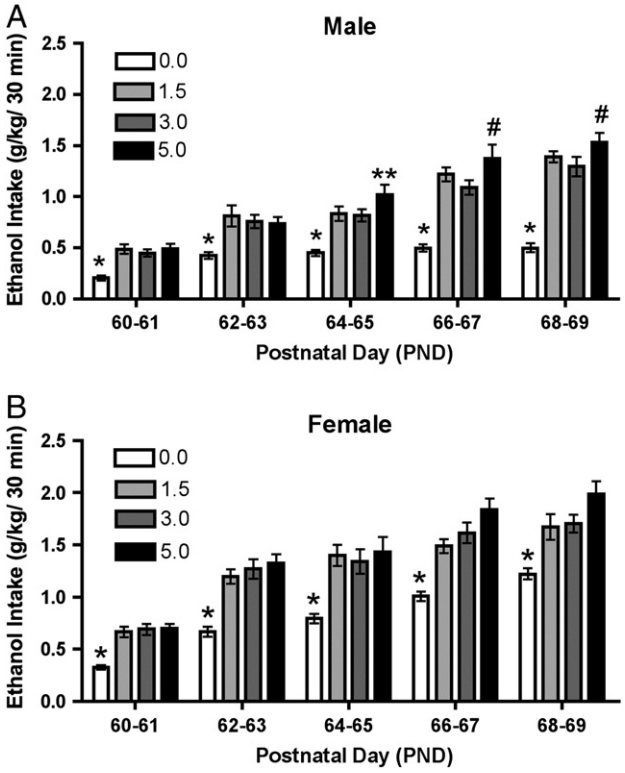

Fig. 1. Mean ± SEM voluntary sweetened EtOH consumption expressed as grams of EtOH consumed per kilogram of body weight (g/kg) across days in young adulthood (PND 60–69) using a limited-access (30 min/day) two-bottle choice paradigm in a holding cage in male (Panel A) and female (Panel B) rats. Panel A) * Water treated rats (0.0 g/kg) significantly lower than 1.5, 3.0 and 5.0 g/kg EtOH-treated rats. # 5.0 g/kg EtOH-treated rats significantly greater than 3.0 g/kg EtOH-treated rats. ** 5.0 g/kg EtOH-treated rats significantly greater than 1.5 and 3.0 g/kg EtOH-treated rats. Male: 0.0 n = 33, 1.5 n = 13, 3.0 n = 12, 5.0 n = 12. Female: 0.0 n = 34, 1.5 n = 10, 3.0 n = 10, 5.0 n = 12.

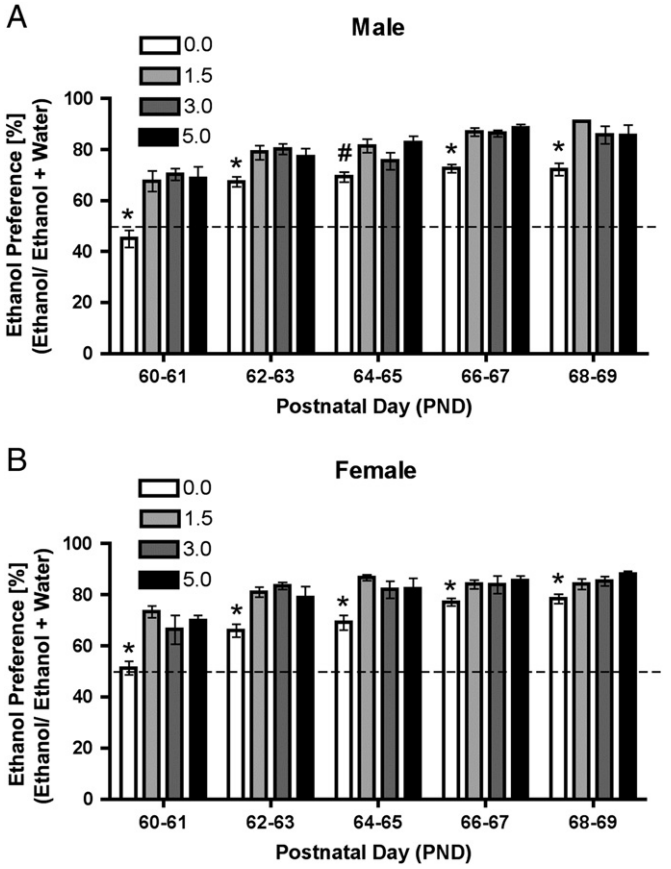

Fig. 2. Mean ± SEM preference (%) ({EtOH (mL)/ [EtOH (mL) + water (mL)]} * 100) for sweetened EtOH across days in young adulthood (PND 60–69) using a limited-access (30 min/day) two-bottle choice paradigm in a holding cage in male (Panel A) and female (Panel B) rats. The dashed line indicates a preference of 50%. Any values higher than 50% indicate a preference for ethanol over water. * Water treated rats (0.0 g/kg) significantly lower than 1.5, 3.0 and 5.0 g/kg EtOH-treated adolescent rats. # 0.0 g/kg EtOH-treated rats significantly lower than 1.5 and 5.0 g/kg EtOH-treated rats. Male: 0.0 n = 33, 1.5 n = 13, 3.0 n = 12, 5.0 n = 12. Female: 0.0 n = 34, 1.5 n = 10, 3.0 n = 10, 5.0 n = 12.

adolescence across days, with the exception of the 3.0 g/kg dose not being significant on PND 64–65 [F (4, 264) = 28.93, $p<0.0005$]. For females, the same pattern of significantly greater preference in young adulthood for EtOH in rats exposed to any dose of EtOH during adolescence relative to water controls {Dose [F (3, 62) = 24.40, $p<0.0005$]} across all days [F (4, 248) = 23.09, $p<0.0005$; Fig. 2B].

Given sex differences were observed in control rats, with females consuming significantly more sweetened EtOH than males when data were expressed as g/kg, data were transformed and expressed as a percent of their respective sex control [(EtOH-treated mean/ water-treated mean) * 100]. A significant two-way interaction of Sex by Days [F (4, 252) = 18.27, $p<0.0005$] and significant main effects of Dose [F (2, 63) = 4.86, $p<0.05$], Sex [F (1, 63) = 75.63, $p<0.0005$] and Days [F (4, 252) = 6.59, $p<0.0005$] were found. The overall three-way ANOVA of Sex by Days by Dose did not reach statistical significance. However, given the Sex by Days main effects and interactions were statistically significant and there were sex differences observed in the control animals; data were analyzed using a two-way ANOVA for Sex by Days for each dose. As depicted in Fig. 3A, when data were equated for innate sex differences in voluntary sweetened EtOH intake, males administered 1.5 g/kg EtOH during adolescence showed a significantly greater relative increase in sweetened EtOH consumption as compared to females {Sex by Days [F (4, 84) = 6.83, $p<0.0005$]} on PND 66–67 {Sex [F (1, 21) = 36.47, $p<0.0005$]} and PND 68–69 {Sex [F (1, 21) = 86.89, $p<0.0005$]}. As shown in Fig. 3B, male rats exposed to 3.0 g/kg EtOH during adolescence exhibited the same pattern of greater relative EtOH consumption {Sex by Days [F (4, 80) = 6.50, $p<0.0005$]} on PND 66–67 [F (1, 20) = 12.00, $p<0.005$] and PND 68–69 [F (1, 20) = 32.53, $p<0.0005$]. Male rats exposed to 5.0 g/kg EtOH during adolescence showed greater relative increases in sweetened EtOH intake in young adulthood as compared to female rats on PND 66–67 [F (1, 22) = 10.13, $p<0.005$] and PND 68–69 [F (1, 22) = 50.94, $p<0.0005$] as supported by the significant two-way interaction of Sex by Days [F (4, 88) = 6.22, $p<0.0005$; Fig. 3C].

3.3. Experiment 2

3.3.1. Weight

To examine changes in weight gain during adolescence and adulthood in male and female rats treated with EtOH relative to water, data were analyzed separately for each sex using a two-way ANOVA for Days and Dose during adolescence and adulthood (data are depicted in bottom left panel in Table 1). For males, a significant two-way interaction of Dose by Days [F (11, 220) = 5.88, $p<0.0005$] and a significant main effect of Days [F (11, 220) = 1828.00, $p<0.0005$] were found during adolescence (PND 28–31, PND 35–38 and PND 42–45). Post-hoc analyses indicate there were no significant differences in weight on any given day during EtOH pretreatment during adolescence. However, control rats showed significant increases in weight gain from PND 30–38 and from PND 43–44

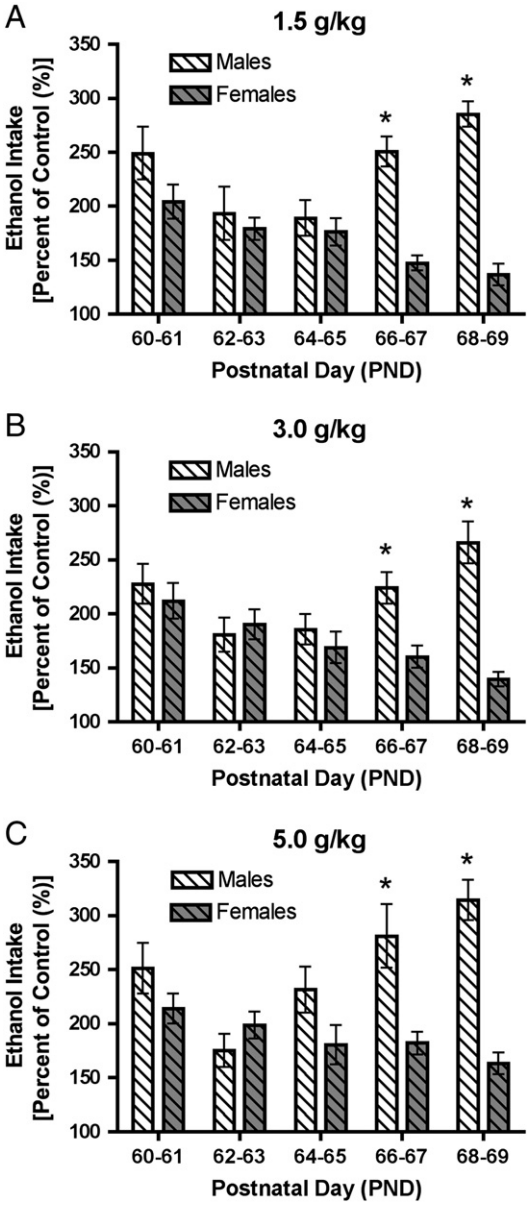

Fig. 3. Mean ± SEM voluntary sweetened EtOH consumption expressed as percent of control in EtOH-pretreated rats equated relative to control (100%) across days in adulthood (PND 60–69) using a limited-access (30 min/day) two-bottle choice paradigm in a holding cage. * indicates male EtOH intakes are significantly greater than females'. Male: 1.5 n = 13, 3.0 n = 12, 5.0 n = 12. Female: 1.5 n = 10, 3.0 n = 10, 5.0 n = 12.

[F (11, 119) = 58.41, p<0.0005], while rats treated with 5.0 g/kg EtOH during adolescence only showed significant increases in weight gain from PND 36–38 [F (11, 143) = 46.09, p<0.0005]. The main effect of Dose failed to reach statistical significance (p>0.05). For females, during adolescent pretreatment, the two-way interaction of Dose by Days and the main effect of Dose failed to reach statistical significance (p>0.05). However, a significant main effect of Days [F (11, 209) = 1352.00, p<0.0005] was revealed.

In young adult males (PND 60–69), a significant main effect of Days [F (9, 180) = 389.30, p<0.0005] was found. The two-way interaction of Dose by Days (p>0.05) and main effect of Dose failed to reach statistical significance (p>0.05). In young adult females (PND 60–69), the two-way interaction of Dose by Days (p>0.05) and the main effect of Dose (p>0.05) failed to reach statistical significance. However, a significant main effect of Days [F (9, 171) = 145.8, p<0.0005] was found.

3.4. Voluntary Saccharin intake

Overall, females consumed significantly more saccharin relative to males as supported by a significant main effect of Sex [F (1, 39) = 17.88, p<0.0005] and a Sex by Days interaction [F (4, 156) = 5.94, p<0.0005; Fig. 4]. EtOH pretreatment during adolescence did not alter voluntary saccharin consumption in young adulthood relative to controls for males {Days by Dose [F (4, 80) = 0.12, p>0.05; Fig. 4A} or females {Days by Dose [F (4, 76) = 0.28, p>0.05; Fig. 4B}. When data were equated as percent of control, the two-way interaction for Sex by Days, and the main effect of Sex and Days all failed to reach statistical significance, indicating no relative differences in voluntary saccharin consumption in young adulthood between EtOH pretreated males and females (data not shown). Female rats treated with water (0.0 g/kg) during adolescence consumed significantly more saccharin as compared to male controls on PND 66–67 and PND 68–69. Female rats treated with 5.0 g/kg during adolescence consumed significantly more saccharin as compared to their male counterparts on PND 60–61, PND 64–65, PND 66–67 and PND 68–69. As shown in panel A, there were no significant differences in voluntary saccharin consumption in young adulthood between male rats pretreated with water (0.0 g/kg) or 5.0 g/kg EtOH during adolescence. As shown in panel B, there were no significant differences in voluntary saccharin consumption in young adulthood between female rats pretreated with water (0.0 g/kg) or 5.0 g/kg EtOH during adolescence.

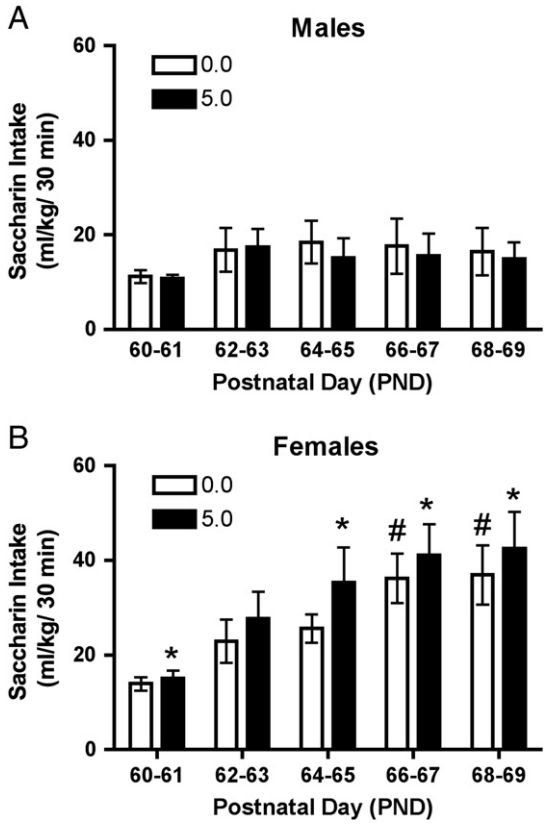

Fig. 4. Mean ± SEM voluntary saccharin consumption expressed as ml of saccharin consumed per kilogram of body weight (ml/kg) across days in young adulthood (PND 60–69) using a limited-access (30 min/day) two-bottle choice paradigm in a holding cage in male (Panel A) and female (Panel B) rats. * 5.0 g/kg EtOH-treated female significantly greater than male 5.0 g/kg-treated counterpart. # Female 0.0 g/kg significantly greater than male 0.0 g/kg counterpart. Male: 0.0 n = 10, 5.0 n = 12. Female: 0.0 n = 12, 5.0 n = 9.

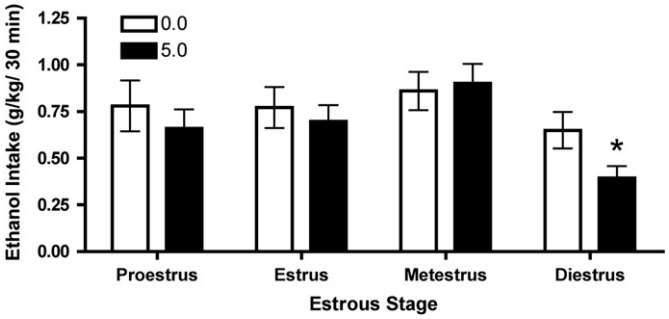

Fig. 5. Mean ± SEM voluntary ethanol consumption expressed as grams of EtOH consumed per kilogram of body weight (g/kg) as a function of stage in the estrous cycle in adulthood (PND 72–81) using a limited-access (30 min/day) two-bottle choice paradigm in a holding cage. * indicates Diestrus is less than Proestrus and Metestrus. 0.0 n = 10, 5.0 n = 10.

3.5. Experiment 3

3.5.1. Weight

To examine changes in weight gain during adolescence and adulthood in female rats treated with EtOH relative to water, data were analyzed using a two-way ANOVA for Days and Dose during adolescence and adulthood (data are depicted in bottom right panel in Table 1). During adolescent pretreatment, the two-way interaction of Dose by Days and the main effect of Dose failed to reach statistical significance ($p>0.05$). However, a significant main effect of Days [$F(11, 198)=1640.00$, $p<0.0005$] was revealed. In adult females (PND 72–81), the two-way interaction of Dose by Days ($p>0.05$) and the main effects of Dose ($p>0.05$) and Days ($p>0.05$) failed to reach statistical significance.

3.6. Estrous stage and voluntary ethanol consumption

In general, adolescent EtOH pretreatment and stage of estrous had an impact on EtOH intake in adulthood in female rats. Voluntary sweetened EtOH intake was differentially affected in adulthood during diestrus (Fig. 5) when data were analyzed with Estrous Stage nested within Dose [$F(7, 72)=2.41$, $p<0.05$]. There were no significant differences in EtOH intake across the estrous cycle in females treated with water during adolescence and tested for voluntary EtOH intake in adulthood ($p>0.05$). In contrast, females treated with 5.0 g/kg EtOH during adolescence showed decreased voluntary EtOH consumption during diestrus in adulthood [$F(3, 36), = 5.46$, $p<0.005$]. A representative sample of each estrous stage are shown in Fig. 6.

4. Discussion

One of the most common methods used in investigating binge pattern EtOH consumption in rodents has been a four-day binge model, used particularly because of its similarity to a model of a "bender" for an alcoholic in humans (Nixon and Crews, 2002). Using this model of EtOH administration to produce tolerance and dependence to EtOH in adult rats, binge EtOH exposure decreased neurogenesis in the adult rat hippocampus (Nixon and Crews, 2002). Moreover, the four-day binge EtOH exposure model induced cognitive dysfunction in rodents, which has been suggested to induce comparable problems in humans (Obernier et al., 2002). Alternatively,

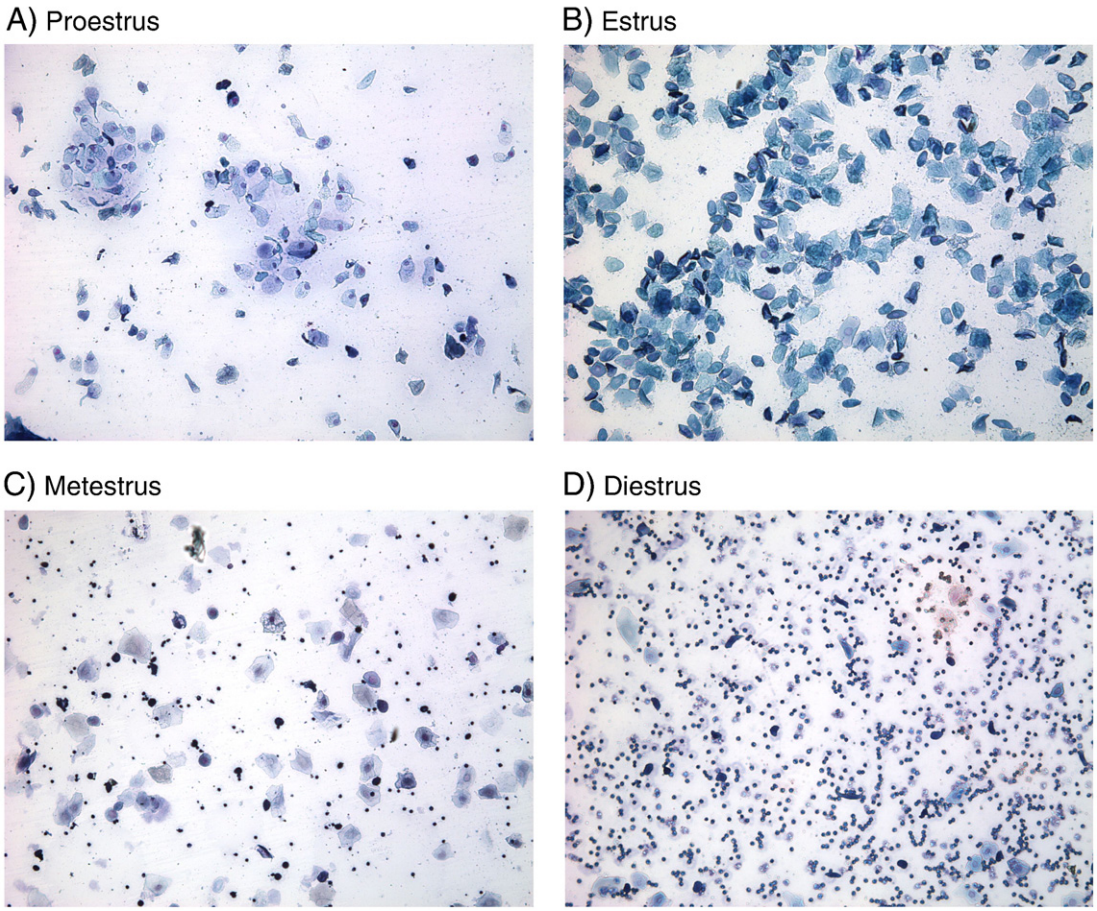

Fig. 6. Representative sample of each stage in the estrous cycle. Panel A) Proestrus: characterized by a predominance of nucleated epithelial cells. Panel B) Estrus: characterized by a predominance of anucleated cornified cells. Panel C) Metestrus: characterized by an equal proportion of nucleated epithelial cells, anucleated cornified cells and leukocytes. Panel D) Diestrus: characterized by a predominance of leukocytes. All definitions are adapted from Marcondes et al., 2002.

using a repeated three-day binge EtOH administration model, adolescent rats repeatedly administered EtOH over four weeks displayed significantly increased anxiety in a passive avoidance task (Popović et al., 2004). While previous work has been conducted to examine the consequences of the four-day binge model (Gavaler et al., 1993; Obernier et al., 2002; Penland et al., 2001), and others have examined the immediate consequences of repeated three-day binge EtOH administration (Popović et al., 2004), there is little research aimed at investigating the long-term behavioral consequences of repeated binge EtOH exposure during adolescence. The pattern of repeated binge EtOH administration during adolescence may serve as an approximate of adolescent human patterns of binge alcohol drinking.

Cognitive and behavioral dysfunctions have been reported following a four-day or repeated three-day binge alcohol model (Popović et al., 2004; Obernier et al., 2002), but previous work had not examined the long-term effects of adolescent models of EtOH exposure in males and females. Overall, data from the present set of experiments demonstrate that all rats exposed to EtOH during adolescence are susceptible to enhanced EtOH consumption in young adulthood, and adolescent females exposed to EtOH subsequently consumed more EtOH (g/kg) in young adulthood than their male counterparts, however this effect was not observed when data were examined as relative to control-treated animals. These data would indicate that male rats treated with EtOH during adolescence are more susceptible to greater EtOH consumption in adulthood following treatment with EtOH rather than water during adolescence using a binge pattern of exposure. Moreover, while no differences in water intake were observed in young adulthood between EtOH-pretreated and control rats in the present set of experiments, EtOH-pretreated rats showed increased preference for sweetened EtOH solution in young adulthood as compared to controls. Furthermore, regardless of EtOH or water pretreatment, no differences were found in EtOH preference over water between males and females in young adulthood. These results suggest a unique vulnerability to a specific pattern of EtOH exposure that induced long-term behavioral changes in adolescent EtOH-exposed versus adolescent EtOH naive rats, and also highlight differences in EtOH consumption between male and female rats.

It is important to note that some forms (EtOH vapor or constant voluntary access to EtOH) of adolescent exposure to EtOH may not induce alterations in voluntary EtOH consumption in adulthood. When EtOH intake was assessed in adulthood, rats given voluntary access to saccharin sweetened or unsweetened EtOH beginning in adolescence and extending into adulthood (PND 28–90) drank similar amounts as rats not given free-access to EtOH until adulthood (PND 71–90; Vetter et al., 2007). In another study, forced periadolescent (PND 30–40) exposure to EtOH vapor for 12 h a day did not enhance sucrose sweetened EtOH drinking in adulthood (>PND 92; Slawecki and Betancourt, 2002). However, in the present set of experiments, adolescents repeatedly exposed to EtOH in a binge fashion displayed a dramatic increase in voluntary saccharin sweetened EtOH intake across days in young adulthood. Specifically, males administered the 1.5, 3.0, and 5.0 g/kg EtOH doses during adolescence showed increases in voluntary EtOH intake across days in young adulthood as compared to stable adulthood EtOH intake in water-treated males. Similarly, females exposed to these EtOH doses exhibited greater absolute EtOH consumption (g/kg of bodyweight) in adulthood compared to controls and their respective male counterparts. One of the key aspects hypothesized to increase EtOH intake in young adulthood was the pattern of adolescent EtOH exposure with repeated cycles of four consecutive days of EtOH administration coupled with intermittent abstinence days during the adolescent exposure period. Vetter and colleagues (2007) allowed animals EtOH access everyday beginning in adolescence through adulthood, with no EtOH-free days. Slawecki and Betancourt (2002) exposed adolescent male rats to EtOH for ten consecutive days, with no EtOH-free days. While adolescent rats exposed to EtOH every day did not show enhanced EtOH consumption in adulthood, rodents exposed to intermittent EtOH vapor during periadolescence exhibited a smaller conditioned taste aversion in adulthood as compared to those exposed to chronic EtOH vapor during periadolescence (Diaz-Granados and Graham, 2007). Given differences in behavioral responses to intermittent EtOH exposure were observed (Diaz-Granados and Graham, 2007), but not when animals were exposed to chronic EtOH during adolescence (Slawecki and Betancourt, 2002; Vetter et al., 2007), it is likely the intermittent nature of the binge exposure used in the present set of experiments induced the behavioral changes observed in response to EtOH in young adulthood in both male and female rats.

Intermittent exposure to EtOH has been shown to enhance EtOH consumption in adolescent rats relative to those given continuous access (Hargreaves et al., 2009). Thus the intermittent nature of EtOH exposure during adolescence likely induced greater EtOH consumption in young adulthood in both male and female rats relative to animals treated with water during adolescence and given access to sweetened EtOH in young adulthood. Alternatively, the alcohol deprivation effect (ADE) of two weeks without EtOH administration could account for the greater EtOH consumption in young adulthood in both male and female rats, consistent with previous work conducted in male and female rats (Fullgrabe et al., 2007; Siegmund et al., 2005). An additional explanation for the results obtained is the impact of repeated EtOH withdrawals during adolescent pretreatment on subsequent EtOH consumption in young adulthood as observed in Experiment 1. Males and females respond differently to EtOH withdrawal, and Devaud and Alele (2004) report that their EtOH administration paradigm of liquid diet likely includes repeated withdrawal, which results in different NMDA receptor composition and may mediate the sex differences observed from exposure to and withdrawal from EtOH. However, this effect is likely not mediated by anxiety, as pretreatment with the high doses of EtOH occurred during adolescence, and sex differences in anxiety during acute withdrawal have been observed in adult males, but not adult female or adolescent male and female rats (Varlinskaya and Spear, 2004).

In Experiment 2, adolescent rats pretreated with EtOH, as compared to those treated with water, did not show increased consumption of the saccharin solution (ml/kg) nor a significantly greater preference for the saccharin solution in young adulthood. Given there was stable voluntary saccharin consumption observed across days in water-treated and EtOH-pretreated males, and stable sweetened EtOH consumption was also observed across days in water-treated males, the greater sweetened EtOH intake observed across days in the adolescent-EtOH treated rats was likely not solely attributed to the sweetener used. In females, given there was an increase in both voluntary saccharin and sweetened EtOH intake observed across days in water (except PND 62–63 in Experiment 2) and EtOH-treated rats, the pattern of greater consumption across days may be attributed to the sweetener used in the solution. However, the significantly greater sweetened EtOH intake observed across days in EtOH-treated females is likely not merely attributed to the sweetener, given there were no significant differences in voluntary saccharin consumption across days in young adulthood between adolescent water- and EtOH-treated female rats.

Consistent with previous work (Almeida et al., 1998; Blanchard et al., 1993; Eriksson and Pikkarainen, 1968; Hutchins et al., 1981; Juarez and Barrios de Tomasi, 1999; Lancaster et al., 1996; Lancaster and Spiegel, 1992; Li and Lumeng, 1984; Walker et al., 2008), in the present set of experiments, females consumed more EtOH than males, but when EtOH intake is presented as a percentage of the control the males drank more than the females. Previous research using neonatal estrogenization as a means to phenotypically masculinize the female rat brain strongly suggests observed sex differences in EtOH intake may be a result of the organization of the

brain during early development, with neonatally-estrogenized female rats and intact male rats consuming significantly less EtOH than intact female rats (Goy and McEwen, 1980; Patchev et al., 1995). These findings suggest, in the present study, the elevated levels of EtOH intake observed by females may be at least partially due to innate differences in the female rat brain. Another possible explanation for the sex differences observed in sweetened EtOH consumption may be due to the fact that female rats may have been less sensitive than male rats to the sedative and hypnotic effects of EtOH as they progressed into young adulthood (Cha et al., 2006; Silveri and Spear, 1998). This may, in turn, have allowed female rats to ingest more EtOH before experiencing feedback that would limit intake. Alternatively, greater saccharin consumption in females may be predictive of greater EtOH consumption, as previous work has shown rats with greater EtOH consumption demonstrate greater saccharin preference (Gosnell and Krahn, 1992) and females show greater mood altering effects of sweets relative to males (Kampov-Polevoy et al., 2004).

Interestingly, when examining EtOH intake after equating male and female controls, males consumed more EtOH than females. These data suggest that EtOH pretreatment during adolescence produced a greater increase in EtOH consumption in young adulthood in males, relative to water-treated controls, than similarly treated females. Thus, males may be more sensitive to the long-term effects of adolescent EtOH treatment on sweetened EtOH intake in young adulthood. While this observed effect would seem counterintuitive relative to the absolute EtOH consumption levels expressed as g/kg of bodyweight, it may be important when examining the differential effects of EtOH in males and females. For example, sex differences regarding the actions of EtOH on internal organs have been observed in animal models. In male rats, chronic EtOH consumption caused a thinning of the ventricular wall and intraventricular septum, resulting in the loss of heart mass (Vary et al., 2006). Chronic EtOH consumption in female rats did not cause this same effect, indicating sex-related differences in peripheral actions of EtOH, with males showing more deleterious effects of EtOH on peripheral organs (Vary et al., 2006). However, female rats were shown to have more severe EtOH-induced injuries than males, including various forms of liver damage (Nanji et al., 2001). Taken together, these data indicate males and females differ in their behavioral responses to EtOH, and the effects of EtOH are differentially regulated through both the central and peripheral nervous systems. Although adolescent EtOH exposure appears to have a greater impact on increasing EtOH consumption in males as compared to females when EtOH intake was equated relative to water-treated controls, the overall greater level of absolute EtOH consumption in females (g/kg of bodyweight) may result in a greater impact to the central and peripheral nervous systems. Thus, it is possible females may, in turn, be more susceptible to the harmful effects of EtOH. It is interesting to note across days in females there was an increase in absolute EtOH intake (Fig. 1: g/kg) and a decrease in the relative difference in EtOH intake (Fig. 3: % of control). This may likely be due to sex differences in ADE and stress responsivity. When adolescent females were subjected to a 14 day ADE, there was a 250% increase over baseline drinking which rapidly declined to approximately 125% over four days, and in response to forced swim stress adolescent females showed a maximal 250% increase above baseline drinking (Fullgrabe et al., 2007). In contrast, when adolescent males underwent a 14 day ADE they showed less than 200% increase over baseline drinking which declined to 100% over 4 days, and in response to forced swim stress adolescent males showed a maximal 150% increase above baseline drinking (Siegmund et al., 2005). Alternatively, the differences in EtOH consumption may be accounted for by alterations in preference for saccharin. Human females have been shown to be more sensitive to the mood altering effects of sweets (Kampov-Polevoy et al., 2004) and rats that show greater alcohol consumption also show greater preference for a saccharin solution (Gosnell and Krahn, 1992). Therefore, the trend for greater saccharin consumption in females may be predictive of the greater EtOH consumption relative to body weight observed in female rats.

Previous research has shown dose-dependent differences due to adolescent (Crews et al., 2006) and adult (Matthews et al., 2002) EtOH exposure. Periadolescent rodents exposed to different doses of EtOH (1.0, 2.5, and 5.0 g/kg) exhibited decreased neural progenitor cell proliferation and neurogenesis that was directly proportional to the dose of EtOH administered (Crews et al., 2006). In another experiment, adult rats administered doses of 1.0, 1.5, and 2.0 g/kg EtOH showed impaired spatial memory performance in a dose-dependent manner in the Morris water task (Matthews et al., 2002). In the present study, while adolescent males and females exposed to EtOH subsequently consumed more EtOH in young adulthood than controls, adolescent females exposed to the highest dose of EtOH, 5.0 g/kg, exhibited the greatest level of EtOH consumption in young adulthood. Notably, males administered the 5.0 g/kg dose during adolescence consumed more EtOH in young adulthood than males treated with the lower EtOH doses. These findings indicate that while adolescent binge exposure to any dose of EtOH caused a significant increase in voluntary EtOH consumption in young adulthood, higher EtOH doses produced an even greater effect. Further studies should explore the impact of lower doses than those used in these experiments (<1.5 g/kg of EtOH) that produce elevations in young adulthood and adulthood EtOH ingestion.

Interestingly, after examining the weights of all rats through adolescence and young adulthood, notable differences in weight gain were discovered. Male EtOH-treated rats, as compared to controls, did not exhibit a significant daily increase in weight across days. However, it is important to note their overall weight on any given day was similar to controls. In contrast, female rats treated with 5.0 g/kg EtOH during adolescence were found to weigh significantly less than their controls (water-pretreated in adolescence and EtOH in young adulthood). These findings may be attributed to a high amount of EtOH or saccharin in the stomach altering normal patterns of caloric intake. Future experiments assessing the subsequent effects of EtOH administration should monitor weight during the EtOH exposure period by yoking the weight of control rats to the mean weight of EtOH-treated rats via controlled feeding (Silvers et al., 2003; Tokunaga et al., 2006). It is possible the differences observed in EtOH intake in adulthood are due solely to greater caloric need among EtOH-pretreated animals. However, it is important to recognize the transient differences in EtOH intake across days in both male and female rats, and therefore caloric need would not be expected to be the only mediating factor for the greater EtOH intake observed in EtOH-pretreated animals.

In the present study, the effects of early exposure to EtOH were specifically observed because adolescence is the developmental period in which alcohol consumption is usually initiated (Kandel et al., 1992), and the effects have not been examined in young adulthood or adulthood. Similarly-treated adult comparison groups were not included in the present study; therefore, it is not clear if the effects observed would be similar in adult EtOH-exposed animals. However, this seems unlikely given adult rats have been found to be more sensitive to the sedative and hypnotic effects of EtOH (Silveri and Spear, 1998). Thus, this increased sensitivity to EtOH's post-ingestive effects may serve to limit the amount of EtOH consumed by adult rats (Slawecki and Samson, 1997). Moreover, voluntary ingestion of sweetened EtOH was specifically assessed in young adulthood because recent findings have shown a pattern of increased problem drinking during this period (Barnes et al., 1992; Grant et al., 1998; Harford, 1993). Research has found an alarming 34% of young adults, in particular, engage in heavy or episodic drinking (Johnston et al., 1993). In the present study, adolescent EtOH-treated rats, as compared to controls, showed a progressive increase in voluntary EtOH intake during young adulthood that suggests further

continuation into later adulthood. In Experiment 3, females were treated with EtOH during adolescence and assessed for EtOH intake later (PND 72–81) than in Experiments 1 or 2. There were no significant differences in EtOH intake when animals were assessed at this older age. These data indicate there is a sensitive period to observe increased EtOH intake in young adulthood, and if animals are not assessed until after PND 69, the increased EtOH intake in EtOH-pretreated animals would not be observed. However, it is likely that if animals were assessed beginning in early adulthood at PND 60, the increased EtOH intake would be maintained.

In Experiment 3, the impact of the estrous cycle on EtOH consumption in female rats was examined. In this experiment, females were repeatedly lavaged from PND 60–71 before the assessment of voluntary EtOH intake in adulthood (PND 72–81). While prior research has shown that the estrous cycle may have an effect on EtOH intake in female rats (Forger and Morin, 1982), it should be noted that multiple studies have found the estrous cycle to have no significant impact on EtOH consumption in female rats (Ford et al., 2002; Roberts et al., 1998). In Experiment 3, there was no change in EtOH consumption observed across the estrous cycle in water-pretreated rats, which is consistent with previous work (Ford et al., 2002; Roberts et al., 1998). In contrast, in females treated with 5.0 g/kg EtOH during adolescence and assessed for voluntary EtOH intake in adulthood, there was a decrease in EtOH consumption during the diestrus phase of the estrous cycle. These data would indicate females treated with this high (5.0 g/kg/ig) dose of EtOH during adolescence altered voluntary EtOH intake in adulthood as a function of the estrous cycle. However, given no differences in voluntary EtOH consumption in females treated with water or 5.0 g/kg EtOH during adolescence in Experiment 3 were observed, which were assessed for voluntary EtOH intake at a later age (PND 72–81) relative to experiments 1 and 2 (PND 60–69), it is difficult to interpret these data beyond a period of vulnerability of heightened EtOH consumption during young adulthood (PND 60–69) relative to later adulthood (PND 72–81). It is possible the repeated lavage prior to assessment of voluntary EtOH intake may have altered EtOH consumption in the females in Experiment 3.

Further, blood EtOH concentrations were not assessed in the present set of experiments. However, given that limited access to EtOH may increase the amount of EtOH consumed per bout (Marcucella and Munro, 1987) and, in turn, leads to greater blood EtOH concentrations per bout (Files et al., 1994), the enhanced levels of EtOH intake observed in the present set of experiments during the 30-min EtOH access session likely induced high blood EtOH concentrations. These blood EtOH concentrations may be important to note, as chronic intermittent injections of high-dose EtOH during adolescence has been found to induce long-lasting tolerance to EtOH as measured by enhanced blood EtOH elimination rates in adulthood compared to saline-treated rats (Silvers et al., 2003). Therefore, when exploring the long-term consequences of adolescent EtOH exposure on subsequently elevated EtOH consumption, future studies should assess the possibility of metabolic EtOH tolerance by examining blood EtOH levels in adulthood.

Considering adolescence is a developmental period in which EtOH is initially consumed and may lead to greater alcohol consumption later in life (Grant et al., 2001; Hasin and Glick, 1992; McCarty et al., 2004; Robin et al., 1998), and the trend of alcoholism among women has grown (de Lint and Schmidt, 1971; Grant and Harford, 1991; Wilsnack and Wilsnack, 1995), the results of the present set of experiments demonstrate the importance of elucidating the impact of early EtOH exposure on the subsequent predisposition to drink later in life. It is possible early patterns of drinking (binge-drinking), rather than simple exposure to EtOH during adolescence, may play a crucial role in the development and continuation of EtOH use disorders into adulthood (Hill et al., 2000). The differences in saccharin consumption in females relative to males may have mediated some of the differences in voluntary EtOH consumption observed in the present set of experiments, given that in humans females have been found to be more sensitive to the effects of sweets relative to males (Kampov-Polevoy et al., 2006). Additionally, age of assessment for voluntary EtOH consumption and the estrous cycle in adolescent EtOH-treated animals also influence the level of voluntary EtOH consumption. Future work should not only further investigate the behavioral and neural mechanisms that mediate sex differences in EtOH consumption, but also examine the biological mechanisms related to the long-term consequences of early EtOH administration on subsequently elevated patterns of EtOH ingestion in adulthood.

Acknowledgements

The authors would like to thank the helpful comments from the reviewers in the revision of this manuscript. This work was funded by NIAAA AA17667, the University of South Florida (USF) Neuroscience Health Program, the USF College of Arts and Sciences, and the USF Department of Psychology.

References

Acheson SK, Ross EL, Swartzwelder HS. Age independent and dose-response effects of ethanol on spatial memory in rats. Alcohol 2001;23(3):167–75.
Almeida OFX, Shoaib M, Deicke J, Fischer D, Darwish MH, Patchev VK. Gender differences in ethanol preference and ingestion in rats: the role of the gonadal steroid environment. J Clin Invest 1998;101(12):2677–85.
Barnes GM, Welte JW, Dintcheff B. Alcohol misuse among college students and other young adults: findings from a general population study in New York State. Int J Addict 1992;27:917–34.
Blanchard BA, Steindorf S, Wang S, Glick SD. Sex differences in ethanol-induced dopamine release in nucleus accumbens and in ethanol consumption in rats. Alcohol Clin Exp Res 1993;17:968–73.
Bradley KA, Badrinath S, Bush K, Boyd-Wickizer J, Anawalt B. Medical risks for women who drink alcohol. J Fen Internal Med 1998;13:627–39.
Cha YM, Li Q, Wilson WA, Swartzwelder HS. Sedative and GABAergic effects of ethanol on male and female rats. Alcohol Clin Exp Res 2006;30(1):113–8.
Crews FT, Braun CJ, Hoplight B, Switzer RC, Knapp DJ. Binge ethanol consumption causes differential brain damage in young adolescent rats compared with adult rats. Alcohol Clin Exp Res 2000;24(11):1712–23.
Crews FT, Mdzinarishvili D, Kim J, He J, Nixon K. Neurogenesis in adolescent brain is potently inhibited by ethanol. Neuroscience 2006;137:437–45.
de Lint J, Schmidt W. In: Israel Y, Mardones J, editors. The epidemiology of alcoholism. Biological Basis of Alcoholism. New York: John Wiley & Sons, Inc; 1971. p. 423–42.
Devaud LL, Alele P. Differential effects of chronic ethanol administration and withdrawal on gamma-aminobutyric acid type A and NMDA receptor subunit proteins in male and female rat brain. Alcohol Clin Exp Res 2004;28(6):957–65.
Diaz-Granados JL, Graham DL. The effects of continuous and intermittent ethanol exposure in adolescence on the aversive properties of ethanol during adulthood. Alcohol Clin Exp Res 2007;31(12):2020–7.
Doremus TL, Brunell SC, Rajendran P, Spear LP. Factors influencing elevated ethanol consumption in adolescent relative to adult rats. Alcohol Clin Exp Res 2005;29(10):1796–808.
Eriksson K, Pikkarainen PH. Differences between the sexes in voluntary alcohol consumption and liver ADH-activity in inbred strains of mice. Metabolism 1968;17:1037–42.
Files FJ, Lewis RS, Samson HH. Effects of continuous versus limited access to ethanol on ethanol self-administration. Alcohol 1994;11(6):523–31.
Ford MM, Eldridge JC, Samson HH. Microanalysis of ethanol self-administration: estrous cycle phase-related changes in consumption patterns. Alcohol Clin Exp Res 2002;26:635–43.
Forger NG, Morin LP. Reproductive state modulates ethanol intake in rats: effects of ovariectomy, ethanol concentration, estrous cycle, and pregnancy. Pharmacol Biochem Behav 1982;17:323–31.
Fullgrabe MW, Vengeliene V, Spanagel R. Influence of age of drinking onset on the alcohol deprivation effect and stress-induced drinking in female rats. Pharmacol Biochem Behav 2007;86:320–6.
Gavaler J, Smith Jr WI, Van Thiel DH, Rosenblum ER, Deal SR, Allan MJ. "Binge" versus steady drinking: effects on the liver in the ovariectomized rat. Alcohol Clin Exp Res 1993;17(2):355–8.
Gosnell BA, Krahn DD. The relationship between saccharin and alcohol intake in rats. Alcohol 1992;9:203–6.
Goy RW, McEwen BS. Sexual differentiation of the brain. Cambridge, MA: MIT Press; 1980. 223.
Grant BF, Harford TC. Prevalence of DSM-III-R alcohol abuse and dependence. Alcohol Health Res World 1991;15(1):91.
Grant BF, Harford TC, Grigson MB. Stability of alcohol consumption among youth: a national longitudinal survey. J Stud Alcohol 1998;49:253–60.
Grant BF, Stinson FS, Harford TC. Age at onset of alcohol use and DSM-IV alcohol use and dependence: a 12-year follow-up. J Subst Abuse 2001;13(4):493–504.

Harford TC. Stability and prevalence of drinking among young adults. Addiction 1993;88:273–7.
Hargreaves GA, Monds L, Gunasekaran N, Dawson B., McGregor IS. Intermittent access to beer promotes binge-like drinking in adolescent but not adult Wistar rats. Alcohol 2009;43:305–14.
Hasin DS, Glick H. Severity of DSM-III-R alcohol dependence: United States, 1988. Br J Addict 1992;87:1725–30.
Hill KG, White HR, Chung I, Hawkins JD, Catalano RF. Early adult outcomes of adolescent binge drinking: person- and variable-centered analyses of binge drinking trajectories. Alcohol Clin Exp Res 2000;24:892–901.
Hunt PS, Holloway JL, Scordalakes EM. Social interaction with an intoxicated sibling can result in increased intake of ethanol by periadolescent rats. Dev Psychobiol 2001;38 (2):101–9.
Hunt PS, Lant GM, Carroll CA. Ehnhanced intake of ethanol in preweanling rats following interactins with intoxicated siblings. Dev Psychobiol 2000;37(2):90–9.
Hutchins JB, Allen DL, Cole-Harding LS, Wilson JR. Behavioral and physiological measures for studying ethanol dependence in mice. Pharmacol Biochem Behav 1981;15:55–9.
Johnston LD, O'Malley PM, Bachman JG, Schulenberg JE. Monitoring the Future national survey results on drug use. Volume I: Secondary school students (NIH Publication No. 09-7402)Bethesda, MD: National Institute on Drug Abuse; 1975-2008.
Johnston L.D., O'Malley P.M., Bachman J.G. National Survey Results on Drug Use from the Monitoring the Future Study 1993; 1975–1992, Vol. 2, NIH Publication No. 93-3597, Washington: Government Printing Office.
Juarez J, Barrios de Tomasi E. Sex differences in alcohol drinking patterns during forced and voluntary consumption in rats. Alcohol 1999;19:15–22.
Kampov-Polevoy AB, Eick C, Boland G, Khalitoc E, Crews FT. Sweet liking, novelty seeking, and gender predict alcoholic status. Alcohol Clin Exp Res 2004;28(9): 1291–8.
Kampov-Polevoy AB, Alterman A, Khalitov E, Garbutt JC. Sweet preference predicts mood altering effect of and impaired control over eating sweet foods. Eat Beh 2006;7:181–7.
Kandel DB, Yamaguchi K, Chen K. Stages of progression in drug involvement from adolescence to adulthood: further evidence for the gateway theory. J Stud Alcohol 1992;53:447–57.
Lancaster FE, Spiegel KS. Sex differences in pattern of drinking. Alcohol 1992;9:415–20.
Lancaster FE, Brown TD, Coker KL, Elliot 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.
Li TK, Lumeng L. Alcohol preferences and voluntary alcohol intakes of inbred rat strains and the National Institutes of Health heterogeneous stock of rats. Alcohol Clin Exp Res 1984;8:485–6.
Marcondes FK, Bianchi FJ, Tanno AP. Determination of the estrous cycle phases of rats: some helpful considerations. Braz J Biol 2002;62(4):609–14.
Marcucella H, Munro I. Ethanol consumption of free feeding animals during restricted ethanol access. Alcohol Drug Res 1987;7(5–6):405–14.
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 (2):416–21.
Matthews DB, Morrow AL, Tokunaga S, McDaniel JR. Acute ethanol administration and acute allopregnanolone administration impair spatial memory in the Morris water task. Alcohol Clin Exp Res 2002;26(11):1747–51.
McCarty CA, Ebel BE, Garrison MM, DiGiuseppe DL, Christakis DA, Rivara FP. Continuity of binge and harmful drinking from late adolescence to early adulthood. Pediatrics 2004;114(3):714–9.
Monti PM, Miranda R, Nixon K, Sher KJ, Swartzwelder HS, Tapert SF, et al. Adolescence: booze, brains, and behavior. Alcohol Clin Exp Res 2005;29(2):207–20.
Nanji AA, Jokelainen K, Fotouhinia M, Rahemtulla A, Thomas P, Tipoe GL, et al. Increased severity of alcoholic liver injury in female rats: role of oxidative stress, endotoxin, and chemokines. Am J Physiol Gastrointest Liver Physiol 2001;281(6):G1348–56.
Nixon K, Crews F. Binge ethanol exposure decreases neurogenesis in adult rat hippocampus. J Neurochem 2002;83:1087–93.
Obernier JA, White AM, Swartzwelder HS, Crews FT. Cognitive deficits and CNS damage after a 4-day binge ethanol exposure in rats. Pharmacol Biochem Behav 2002;72 (3):521–32.
Pascual M, Blanco AM, Cauli O, Miñarro J, Guerri C. Intermittent ethanol exposure induces inflammatory brain damage and causes long-term behavioural alterations in adolescent rats. Eur J Neurosci 2007;25(2):541–50.
Patchev VK, Hayashi S, Orikasa C, Almeida OF. Implications of estrogen-dependent brain organization for gender differences in hypothalomo–pituitary–adrenal regulation. FASEB 1995;9(5):419–23.
Penland S, Hoplight B, Obernier J, Crews FT. The effects of nicotine on ethanol dependence and brain damage. Alcohol 2001;24:45–54.
Popović M, Caballero-Bleda M, Puelles L, Guerri C. Multiple binge alcohol consumption during rat adolescence increases anxiety but does not impair retention in the passive avoidance task. Neurosci Lett 2004;357(2):79–82.
Roberts AD, Smith AD, Weiss F, Rivier C, Koob GF. Estrous cycle effects on operant responding for ethanol in female rats. Alcohol Clin Exp Res 1998;22:1564–9.
Robin RW, Long JC, Rasmussen JK, Albaugh B, Goldman D. Relationship of binge drinking to alcohol dependence, other psychiatric disorders, and behavioral problems in an American Indian tribe. Alcohol Clin Exp Res 1998;22(2): 518–23.
Siegmund S, Vengeliene V, Singer MV, Spanagel R. Influence of age at drinking onset on long-term ethanol self-administration with deprivation and stress phases. Alcohol Clin Exp Res 2005;29(7):1139–45.
Silveri MM, Spear LP. Decreased sensitivity to the hypnotic effects of ethanol early in ontogeny. Alcohol Clin Exp Res 1998;22:670–6.
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. Alcohol Clin Exp Res 2003;27(10):1606–12.
Silvers JM, Tokunaga S, Mittleman G, O'Buckley T, Morrow AL, Matthews DB. Chronic intermittent ethanol exposure during adolescence reduces the effect of ethanol on hippocampal allopregnanolone levels and Morris water maze task performance. Alcohol 2006;39(3):151–8.
Slawecki CJ, Betancourt M. Effects of adolescent ethanol exposure on ethanol consumption in adult rats. Alcohol 2002;26:23–31.
Slawecki CJ, Samson HH. Changes in oral ethanol self-administration patterns resulting from ethanol concentration manipulations. Alcohol Clin Exp Res 1997;21(6): 1144–9.
Substance Abuse and Mental Health Services Administration. Summary of Findings From the 1999 National Household Survey on Drug Abuse 2001; DHHS Publication No. (SMA) 00-3466. U.S. Department of Health and Human Services, Rockville, MD.
Tokunaga S, Silvers JM, Matthews DB. Chronic intermittent ethanol exposure during adolescence blocks ethanol-induced inhibition of spontaneously active hippocampal pyramidal neurons. Alcohol Clin Exp Res 2006;30:1–6.
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 2004;28(1):40–50.
Vary T, Kimball R, Sumner A. Sex-dependent differences in the regulation of myocardial protein synthesis following long-term ethanol consumption. Am J Physiol Regul Integr Comp Physiol 2006;292:R778–87.
Vetter C, Doremus T, 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(7):1159–68.
Walker BM, Walker JL, Ehlers CL. Dissociable effects of ethanol consumption during the light and dark phase in adolescent and adult Wistar rats. Alcohol 2008;42:83–9.
Wechsler H, Davenport A, Dowdall G, Moeykens B, Castillo S. Health and behavioral consequences of binge drinking in college: a national survey of students at 140 campuses. JAMA 1994;272:1672–7.
Wechsler H, Dowdall GW, Davenport A, Castillo S. Correlates of college student binge drinking. Am J Public Health 1995;85(7):921–6.
Wechsler H, Kuo M, Lee H, Dowdall GW. Environmental correlates of underage alcohol use and related problems of college students. Am J Prev Med 2000;19: 24–9.
Wilsnack SC, Wilsnack RW. Drinking and problem drinking in US women, 12. Recent Developments in Alcoholism; 1995. p. 29–60. Galanter, Penem Publishing Corp, New York.
Wilsnack RW, Vogeltanz ND, Wilsnack SC, Harris TR. Gender differences in alcohol consumption and adverse drinking consequences: cross-cultural patterns. Addiction 2000;95(2):251–65.