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The effects of prepubertal gonadectomy and binge-like ethanol exposure during adolescence on ethanol drinking in adult male and female rats

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Abstract

The pubertal surge in gonadal hormones that occurs during adolescence may impact the long-term effects of early alcohol exposure and sex differences in drinking behavior in adulthood. We investigated this hypothesis by performing sham or gonadectomy surgeries in Long Evans rats around postnatal day (P) 20. From P35–45, males and females were given saline or 3.0 g/kg ethanol using a binge-like model of exposure (8 injections total). As adults (P100), they were trained to self-administer ethanol via a sucrose-fading procedure and then given access to different unsweetened concentrations (5–20% w/v) for 5 days/concentration. We found that during adolescence, ethanol-induced intoxication was similar in males and females that underwent sham surgery. In gonadectomized males and females, however, the level of intoxication was greater following the last injection compared to the first. During adulthood, females drank more sucrose per body weight than males and binge-like exposure to ethanol reduced sucrose consumption in both sexes. These effects were not seen in gonadectomized rats. Ethanol consumption was higher in saline-exposed females compared to males, with gonadectomy reversing this sex difference by increasing consumption in males and decreasing it in females. Exposure to ethanol during adolescence augmented ethanol consumption in both sexes, but this effect was statistically significant only in gonadectomized females. Together, these results support a role for gonadal hormones during puberty in the short- and long-term effects of ethanol on behavior and in the development of sex differences in consummatory behavior during adulthood.

Keywords

adolescence; gonadectomy; ethanol self-administration; sex differences; ovariectomy

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Introduction

Alcohol consumption varies widely by age and sex [28,89]. Approximately 85% of the population in the United States has their first drink by age 21 and the incidence of binge and heavy alcohol use is significantly higher during adolescence compared to adulthood [66]. Sex differences in alcohol intake, with males consuming more and having higher rates of alcohol dependence, are most notable in adulthood [29]. However, examination of drinking patterns in adolescents suggests a role for gonadal steroid hormones in the development of sex differences in drinking behavior. For example, early-maturing girls and boys have been shown to have higher rates of alcohol use [54,83]. Furthermore, early menarche is associated with a higher incidence of drinking and smoking in adolescent girls [16,88]. Studies in rodent models also show that adolescence is a period of increased alcohol consumption compared to adulthood [19,44,85]. Evidence for sex differences in adolescent consumption is somewhat equivocal: adolescent males have been reported to drink more than females in some rodent studies [37,86], whereas others suggest that there is no sex difference [19,82].

Activation of the hypothalamic-pituitary-gonadal axis, which leads to significant increases in circulating estrogen and testosterone, likely facilitates the structural and functional changes in the central nervous system that occur during adolescence [9,40]. This includes volumetric changes in brain areas such as the frontal cortex, cerebellum, hippocampus, and amygdala [24,25,32,45,64,80]. Also evident are changes in the neurotransmitter systems that mediate alcohol-related behaviors, including GABA [49,90], glutamate [12,91], dopamine [2,5], acetylcholine [20,41], and serotonin [17,35]. In rodents, sex-specific drinking patterns coincide with the rise of gonadal hormones during adolescence [18], with females typically consuming higher quantities of alcohol than males post-puberty [19,37,82]. Notably, gonadectomy during adulthood does not significantly influence this sex difference in alcohol intake [1,8]. Thus, rather than modulating alcohol intake directly, gonadal steroids during adolescence may organize mechanisms that lead to sexually disparate alcohol consumption.

Gonadal steroids may also modulate the long-term effects of alcohol when exposure starts during adolescence. In humans, drinking during adolescence is associated with heightened vulnerability to developing alcoholism [27]. This effect is more robust in females, as the duration is shorter between drinking onset and the diagnosis of alcohol dependence [57]. In rodents, adolescent exposure to alcohol has been shown to increase consumption in adulthood for both males and females [6,53,75], though this effect is dependent on factors such as strain [51], exposure procedure [72], and the method of assessing drinking [68,79].

The purpose of the present study was to determine if preventing the rise in gonadal hormones during adolescence would influence sex differences in alcohol (ethanol) consumption during adulthood. Additionally, we determined if this manipulation altered the effects of ethanol exposure during adolescence on drinking behavior in adulthood. Male and female Long Evans rats were gonadectomized before puberty and later, during the peri-adolescent period, we exposed them to saline or ethanol using a binge-like pattern of administration. After animals reached adulthood, they were trained to drink unsweetened ethanol and consumption was measured at various concentrations using a limited-access drinking paradigm.

Materials and Methods

Subjects and surgical procedures

Male (n = 49) and female (n = 60) Long-Evans rats, bred in our animal facility from stock rats obtained from Simonson Labs (Gilroy, CA), were housed in groups of 2–3 rats/cage on a 12 hr light/dark cycle (lights on at 0800 h). They were maintained in a temperature and

humidity controlled room, with food available *ad libitum* throughout the duration of the study. Water was also available *ad libitum*, except for 16 hrs before the first day of the sucrose-fading procedure (see below). All procedures were consistent with the `Principles of Laboratory Animal Care' (NIH Publication no. 85-23) and were approved by the IACUC at the University of Illinois, Urbana-Champaign, USA.

Bilateral gonadectomy (GDX and OVX for males and females, respectively) or sham surgeries were performed on postnatal day (P) 20 ± 1 day. Rats were anesthetized (2.5% isoflurane) and small incisions were made through the skin and intramuscular wall of the ventral torso. The testes or ovaries were removed and the skin was sutured with nylon thread. Sham surgeries were preformed the same way except the gonads were left intact. After the surgery, rats were returned to the dam until weaning on P25.

Binge-like ethanol exposure

From P35–45, rats were exposed intermittently to saline or 3.0 g/kg ethanol (25% v/v) via intraperitoneal (i.p.) injection. The exposure schedule consisted of one injection per day for two consecutive days, followed by one day with no injections. This cycle repeated four times for a total of 8 injections across 11 days. A similar exposure method has been previously shown to produce enduring behavioral and neurophysiological changes that persist into adulthood [53]. A subset of rats in the ethanol-treated groups (n = 24 males and 29 females) were monitored following the first and last ethanol injections using a previously established intoxication rating system [10,43]. For these 60-min sessions, rats were placed individually into a clear plastic enclosure (43×22×20 cm) lined with hardwood bedding. These enclosures and bedding were comparable to those used for the rat's home cage. Observers that were blind to surgery condition scored behavior on a scale from 0–5, with a score of 0 given for no overt signs of intoxication, 1 for general sedation, 2 for mild ataxia and staggering gate, 3 for severe motor impairment, 4 for little to no motor behavior without loss of the righting reflex (LORR), and 5 for LORR, which was defined as an inability of the animal to right itself twice within 30 sec of being placed in the supine position. Intoxication level was assessed at 5, 10, and 15 min post-injection, with the mean of these scores used in subsequent analyses. The total amount of time each animal displayed LORR was also recorded.

Ethanol self-administration in adulthood

When the males (n = 49) and a subset of the females (n = 45) reached P100, they were trained to drink ethanol using a modified sucrose-fading procedure [23,67]. Daily drinking sessions took place in a separate drinking chamber that was comparable to the rats' home cages and the enclosures used during binge-like ethanol exposure. All solutions were presented for 30 min in a single 100 ml bottle fitted with a double ball-bearing sipper tube (Ancare; Bellmore, NY) that minimized leakage. Initially, animals were water deprived for 16 hrs before they were given a session with access to 20% sucrose. They were then returned to *ad libitum* water access in their homecage and given a single session with 20% sucrose. During the next training phase, rats were given two sessions each with a sucrose-ethanol solution that had the concentration of sucrose reduced from 20, 10, 5, and 2.5% over successive sessions while the concentration of ethanol was kept constant at 5% (w/v). Following three sessions of 5% ethanol alone, rats were given two sessions with access to 5% sucrose + 10% (w/v) ethanol, two sessions with access to 2.5% sucrose + 10% ethanol, and three sessions with 10% ethanol alone. Lastly, rats were given two sessions with access to 5% sucrose + 20% (w/v) ethanol, two sessions with access to 2.5% sucrose + 20% ethanol, and three sessions with 20% ethanol alone.

In the next phase of training, a stable baseline of ethanol consumption was determined by providing rats ten daily 30 min sessions with access to 10% ethanol. This was followed by a concentration-response analysis wherein rats were presented with various concentrations of ethanol (5–20%) during their daily 30 min drinking sessions. Ethanol concentrations were presented in random order for 5 consecutive days each. Lastly, rats underwent ten days without drinking sessions (i.e., forced abstinence) and were then given five, once daily 30-min sessions where a single bottle of 10% ethanol was available.

Data analysis

Ratings of intoxication were obtained at 5, 10, and 15 min post-injection on the first and last day of binge-like ethanol exposure (P35 and 45, respectively). The mean of these ratings was taken as each rat's intoxication score. These scores, along with the amount of time each rat displayed LORR, were analyzed using mixed factorial ANOVA with sex (male or female) and surgery (sham or gonadectomy) as the between-subjects factor and injection number (1 or 8) as the repeated measure. A mixed factorial ANOVA (surgery × adolescent exposure × age, with P35, P45, and P100 as the levels of the repeated measure of age) was used to analyze body weights. Sucrose intake in adult males and females was analyzed with between-subjects ANOVA (sex × surgery × adolescent exposure), whereas ethanol intake was analyzed using a mixed factorial ANOVA (sex × surgery × adolescent exposure × ethanol concentration, with 10, 5, 8, 15, 20, and 10% as the levels of the repeated measure of ethanol concentration). Significant interactions were further analyzed with one, two, or three-way ANOVA and Holm-Sidak post-hoc tests.

Results

Binge-like exposure to ethanol during adolescence

Exposure to saline or 3.0 g/kg ethanol began on P35 and continued through P45. As shown in Fig. 1, males and females showed similar levels of intoxication following ethanol treatment at injection 1 and 8. Overall ANOVA revealed a significant interaction between surgery and injection number [$F(1,98) = 4.01$, $p < 0.05$]. This was because gonadectomized rats achieved higher levels of intoxication following the last compared to the first injection [$F(1,51) = 4.52$, $p < 0.05$]. Approximately 60% of rats (30 out of 53) became sufficiently intoxicated to reach LORR following the first or last injection (12.3 ± 2.50 and 13.7 ± 2.74 min, respectively, for all rats regardless of sex or treatment group). However, the duration of LORR in these animals was not statistically different across injection, sex, or surgery, nor were there any significant interactions among these factors.

Body weights during adolescence and adulthood

Prepubertal gonadectomy and ethanol exposure influenced growth rates during the peri-adolescent exposure period, particularly in males. ANOVA revealed a statistically significant interaction between adolescent exposure and age [$F(2,135) = 3.40$, $p < 0.05$]. As shown in Fig. 2, males that were exposed to ethanol during adolescence had significantly reduced weights on P45 compared to saline controls. This was particularly pronounced in GDX males. Nonetheless, in adulthood (P100) there were no significant weight differences between ethanol-exposed and saline-exposed rats. Females' weights were not significantly affected by adolescent ethanol exposure, although there was a trend for female ethanol-exposed rats to weigh less than controls [$F(1,122) = 1.10$, $p = 0.08$]. As expected, prepubertal gonadectomy significantly influenced weights in both sexes. In males, there was a significant interaction between surgery and age [$F(2,135) = 5.84$, $p < 0.01$], with GDX males weighing significantly less than sham controls after reaching P45. There was also a significant interaction between surgery and age in females [$F(2,122) = 23.9$, $p < 0.001$], with OVX females weighing significantly more than intact controls after reaching P45. The

effects of gonadectomy on weight were most pronounced after males and females reached P100.

Ethanol self-administration in adulthood

In order to assess if these adolescent manipulations influenced general consummatory behavior, we first measured intake of a highly palatable 20% w/v sucrose solution, which was offered unadulterated during the early stages of the sucrose-fading procedure. A three-way ANOVA of these data revealed a significant main effect of sex [$F(1,86) = 5.23$, $p < 0.05$] and a significant interaction between surgery and adolescent exposure [$F(1,86) = 5.94$, $p < 0.05$]. As shown in Fig. 3, saline-exposed females that underwent sham surgery consumed more sucrose per body weight than males in this control group. Exposure to ethanol during adolescence reduced sucrose consumption in males and females. The statistically significant sex difference in sucrose consumption, and the effect of ethanol exposure, was not seen in gonadectomized rats. Nonetheless, male and female gonadectomized rats exposed to ethanol did consume significantly more sucrose than sham, ethanol-exposed controls (Fig. 3).

Following the sucrose-fading procedure, rats were given daily access to various concentrations of ethanol ranging from 5–20% w/v. Overall ANOVA revealed a significant main effect of ethanol concentration [$F(5,516) = 29.0$, $p < 0.001$] and a significant sex × surgery × adolescent exposure interaction [$F(1, 516) = 4.93$, $p < 0.05$]. The data for males and females were subsequently analyzed using separate one-way ANOVAs. As shown in Fig. 4, rats tended to achieve the highest level of ethanol intake when presented the higher concentrations of ethanol, with males consuming the most when they were given concentrations over 10% [$F(5,288) = 18.0$, $p < 0.001$] and females likewise escalating intake as ethanol concentration increased [$F(5,264) = 10.4$, $p < 0.001$].

To further assess sex differences in ethanol intake, the data were collapsed across ethanol concentration. This revealed significant interactions of surgery × sex [$F(1,556) = 18.8$, $p < 0.001$] and adolescent exposure × sex [$F(1,556) = 6.85$, $p < 0.01$]. As shown in Fig. 5, males that underwent sham surgery consumed significantly less ethanol than sham females. Sex differences were also evident in response to prepubertal gonadectomy and adolescent ethanol exposure. In adulthood, GDX males consumed significantly more ethanol than shams. OVX females, in contrast, consumed less ethanol than their same-sex sham surgery controls. This effect of gonadectomy in females was reversed by adolescent alcohol exposure. Thus, ethanol exposure during adolescence augmented ethanol consumption in females, but had no significant effect on intake in males. This was true for both surgery types, but statistically significant from saline-exposed rats only in those that underwent OVX surgery.

Discussion

The results of these experiments suggest that preventing the rise in gonadal steroids during puberty alters responses to repeated ethanol exposure during adolescence and has significant effects on consummatory behavior in adulthood. In adolescence, gonadectomized males and females achieved heightened levels of intoxication following the last exposure to ethanol. In adulthood, females consumed more sucrose than males and ethanol exposure during adolescence decreased sucrose consumption in both sexes. Gonadectomy partially reversed these effects of ethanol exposure, with saline- and ethanol-exposed rats of both sexes consuming similar amounts of sucrose. Ethanol consumption during adulthood was also higher in females compared to males and these sex differences were significantly modulated by prepubertal gonadectomy and, to a lesser extent, by adolescent ethanol exposure. GDX

males consumed significantly higher quantities of ethanol than same-sex sham surgery controls, while OVX females consumed less than their controls.

Previous reports indicate that rodents repeatedly exposed to high doses of ethanol during adolescence develop tolerance to the hypothermic, sedative, and hypnotic effects of ethanol [46,78]. Similarly, we found no changes in ethanol intoxication in adolescent male and female rats given binge-like ethanol exposure and sham surgeries. Gonadectomy, however, appeared to enhance ethanol impairment in both sexes following repeated ethanol exposure. Ethanol-induced locomotor impairment is mediated, at least in part, via activation of $GABA_A$ receptors located within the cerebellum [14,77]. Human imaging studies indicate that the cerebellum is one of the brain regions that is still developing during adolescence [52,80]. Rodent studies are consistent with these findings and suggest that gonadal hormones modulate cerebellar development [42,76] and $GABA_A$ function [7]. Furthermore, gonadal hormones are thought to be protective against ethanol-induced neurotoxicity and withdrawal via GABAergic activation [31,34,60,61]. Thus, altered cerebellar development and GABAergic function in gonadectomized rats may contribute to the increased sensitivity to ethanol intoxication we observed here.

Although we found effects of gonadectomy on ethanol-induced intoxication, we did not see differences in LORR duration. In fact, just over half of the rats we tested displayed LORR to the 3.0 g/kg dose of ethanol. Although it is not clear why gonadectomy influenced ethanol-induced locomotor impairment but not LORR, previous reports indicate that adolescents generally display decreased sensitivity to the sedative-hypnotic effects of ethanol [69] and show only modest LORR in response to ethanol doses below 4.0 g/kg [46]. Notably, similar doses (2.0 – 3.0 g/kg) given during a binge pattern of exposure have been shown previously to produce significant blood ethanol concentrations (BECs; ~200 mg%) and lead to long-term behavioral changes and neuroadaptations within the mesocorticolimbic system [53,56] in adolescent rats.

Significant, acute effects of exposure to ethanol on body weight in adolescent rats have been reported previously following an intermittent exposure regimen [70]. In the present study, ethanol treatment-induced decreases in body weight were statistically significant in males, but not in females. We also found no evidence of gross, morphological changes when rats reached adulthood, as the weights of ethanol-exposed rats from both sexes where not different from their saline-exposed controls. Previous studies have suggested that binge-like exposure to ethanol during adolescence can produce long-term effects on bone mass and skeletal development [38,87]. We did not measure these parameters in the current study, but it is unlikely that they played a significant role in adult drinking behavior because we found a greater influence of ethanol exposure during adolescence in the rats that did not exhibit significant ethanol-induced decreases in body weight at P45 (i.e., females). The reduced body weights of males and females that were gonadectomized before puberty, which were expected based on previous studies [73], are indicative of successful removal of sex hormones via the pre-pubertal gonadectomy.

In adulthood, we found that females consumed higher doses of ethanol than males. This finding is consistent with the majority of reports indicating that adult female rats consume greater quantities of ethanol than males per body weight [19,37,39]. Interestingly, this is an effect that tends to emerge following puberty [19,37,82]. Previous studies have demonstrated inconsistent effects of gonadectomy on sex-specific patterns of ethanol intake during adulthood. For example, some studies have shown that adult gonadectomy has little to no effect on male and female rat drinking behavior [1,8], whereas others [4,22] show that ovariectomy reduces ethanol consumption in females. In these studies, the effect of ovariectomy was reduced by subsequent replacement therapy with estradiol, but not

progesterone. In males, gonadectomy in adulthood had no consistent effects on ethanol consumption, but subsequent administration of either testosterone or estrogen increased ethanol drinking under certain conditions [8,30,36]. Differences in strain, ethanol drinking protocol, or age of assessment, all of which varied across these studies, may have contributed to the lack of consistency in these findings, but one factor they all had in common was that gonadectomies were performed after puberty. Here, we addressed whether the sex-specific patterns of ethanol intake in adulthood were influenced by the rising hormone levels during puberty by preventing this hormonal surge through prepubertal gonadectomy. We found that the effects of gonadectomy on drinking behavior persist well into the adult time period, thereby supporting the hypothesis that sex differences in ethanol intake during adulthood are established by gonadal hormones during puberty. Future studies that employ hormone replacement procedures in rats given prepubertal gonadectomy will be important for determining if sex differences in ethanol intake that are blocked by prepubertal gonadectomy can be reversed. Furthermore, they will help identify if puberty is a critical period during which sex differences in drinking behavior emerge. In addition to the issue of timing, further experiments that replace the gonadal steroids both chronically or cyclically are needed to elucidate the role that specific hormones have in the effect.

The mechanisms of the effects of pre-pubertal gonadectomy on ethanol drinking behavior in adulthood are not clear. One factor that potentially contributed to the effects we observed was an influence of early life experience. Early life stress, for example, is well known to alter behavioral outcomes in adulthood [33]. Rats that underwent pre-pubertal gonadectomy were subjected to brief (~ 90 min) maternal separation and, upon their return to the dams, may have been subjected to increased maternal attention (i.e. licking and grooming). However, we controlled for this by using comparison groups that underwent identical procedures except their gonads were left intact. Furthermore, the effects of maternal behaviors on pups are considerably more robust in the early postnatal period compared to just prior to weaning [47,50]. Another potential contributing factor to our observed effects is sex differences in hepatic alcohol dehydrogenase (ADH) activity and ethanol metabolism. These have been reported in humans and rodent models, with females typically showing more rapid ethanol clearance than males [48]. However, the effects of gonadectomy in adulthood on these differences in ethanol metabolism have been inconsistent. In rodents, gonadectomy in males has been shown to produce no change or an increase in hepatic activity and ethanol clearance, whereas ovariectomy had no effect in females [26,59]. Furthermore, ethanol pharmacokinetics in the blood and the brain have been shown to be unaltered across the estrous cycle in female Sprague-Dawley rats [62]. BEC was not measured in the present study, so it is unclear if the effect of gonadectomy on male intake reported here is in part due to changes in ethanol metabolism. However, it is unlikely these effects are solely the result of altered ethanol metabolism because we did not observe any sex, surgery, or treatment group differences in LORR duration. It is also noteworthy that the ethanol doses consumed in the present study are consistent with previous reports of intake in outbred rats tested in limited-access home cage and operant paradigms, where doses and BECs typically range between 0.2–0.8 g/kg and 10–50 mg%, respectively [13,53,82,86].

Because we used a sucrose-fading procedure to engender self-administration of significant amounts of ethanol, we were also able to determine potential group differences in consumption of a natural reward. We found that females drank greater quantities of sucrose when intake is adjusted for body weight. Previously, female rats have been shown to be more responsive to sweet tastants [11,92], which may contribute to the sex difference in sucrose intake shown here. Interestingly, the sex difference in sucrose consumption was abolished by gonadectomy. We also found that adolescent ethanol exposure led to reduced sucrose intake in sham surgery rats of both sexes, but had no effect on gonadectomized animals. This finding suggests gonadectomy may have blocked ethanol-induced `anhedonia'.

In adult rats, chronic ethanol exposure has previously been shown to reduce motivation to procure sucrose under both fixed ratio (FR1) and progressive ratio (PR) schedules of reinforcement [71]. Our findings are consistent with other studies showing that gonadectomy influences reward-related behavior in response to drugs like cocaine [65] and nicotine [81].

We found that exposure to ethanol during adolescence also influenced ethanol intake in adulthood, although this effect was statistically significant only in OVX females. Similar findings in mice have recently been reported showing females to be more sensitive than males to binge-like exposure to ethanol during adolescence [75]. It is not clear why sham females in the current study were not affected in the same robust way as OVX females, although this finding is consistent with other studies suggesting that estrogen is protective against the long-term effects of ethanol exposure during adolescence [60]. Furthermore, adolescent ethanol exposure does not always influence self-administration in adult rats [72] and the effects may appear only under certain conditions (e.g. stress [68]). Meanwhile, others have shown that adolescent ethanol exposure modulates multiple ethanol-induced behaviors in adulthood, including novelty response [74], responsiveness to ethanol odor [21], LORR [46], withdrawal [10], and self-administration [53,63].

Although the mechanisms underlying the effects of early alcohol exposure on drinking behavior in adulthood are not fully understood, one contributing factor may be ethanol-induced disruption of the neurophysiological systems that mediate ethanol consumption. In support of this hypothesis, binge-like exposure to ethanol during adolescence modulates the expression of dopaminergic and glutamatergic receptors in the prefrontal cortex and nucleus accumbens [53]. In addition, recent microdialysis studies indicated that dopaminergic neurotransmission is altered in rats that are exposed to ethanol during adolescence [3,55,56]. Changes in hypothalamic pituitary adrenal (HPA) axis regulation were also found in male and female rats after binge-like ethanol exposure during adolescence [58]. Further studies are warranted in order to assess the role these physiological changes play in the long-term behavioral consequences of adolescent exposure. Alternatively, it is possible that changes in ethanol metabolism may have contributed to the effects we found here. Relatively few studies have assessed changes in ethanol metabolism following repeated exposure during adolescence, with one study demonstrating metabolic tolerance in adolescents twelve days following repeated ethanol exposure [70]. Others have reported no changes in BECs following exposure [51,84].

In summary, our findings highlight the important role that gonadal hormones during puberty play in the acute intoxicating effects of ethanol during adolescence and in sex differences in the consumption of natural rewards (sucrose) and ethanol in adulthood. Future studies will be necessary to determine if it was the lack of hormones during the pubertal period itself that was critical or whether gonadectomy in adulthood would produce the same effect. It is noteworthy, however, that previous studies utilizing gonadectomy in adulthood argue against the latter possibility in regards to gonadectomy effects on ethanol drinking. At the present time, the specific underlying mechanisms of the effects we observed are unclear, but it is possible that gonadal hormones influence ethanol drinking behavior by modulating individual sensitivity to the rewarding, or alternatively the aversive properties, of ethanol [15,84,86]. Future studies are warranted to elucidate if adolescents, relative to adults, are especially sensitive to the long-lasting neurophysiological and behavioral effects of ethanol and how gonadal steroids modulate this heightened sensitivity.

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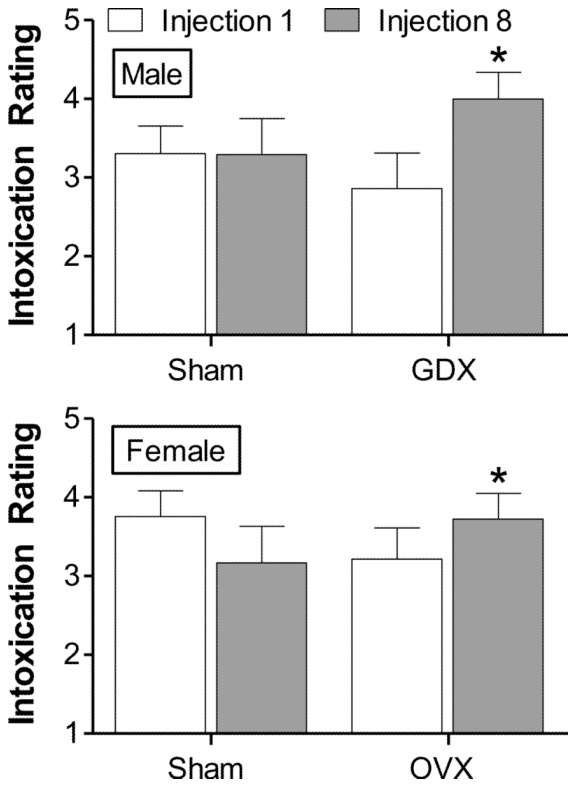

Figure 1.

Intoxication ratings following the first and last injection (1 and 8, respectively) during binge-like ethanol exposure during adolescence. Males (top; n = 24) and females (bottom; n = 29) were rated every 5 min for 15 min following 3.0 g/kg ethanol (i.p.). A maximum score of 5 was given in each bin and the group averages of the mean scores across the 15 min period are shown here (see Methods for a description of the scoring procedure). * $p < 0.05$, compared to injection 1 within the same group.

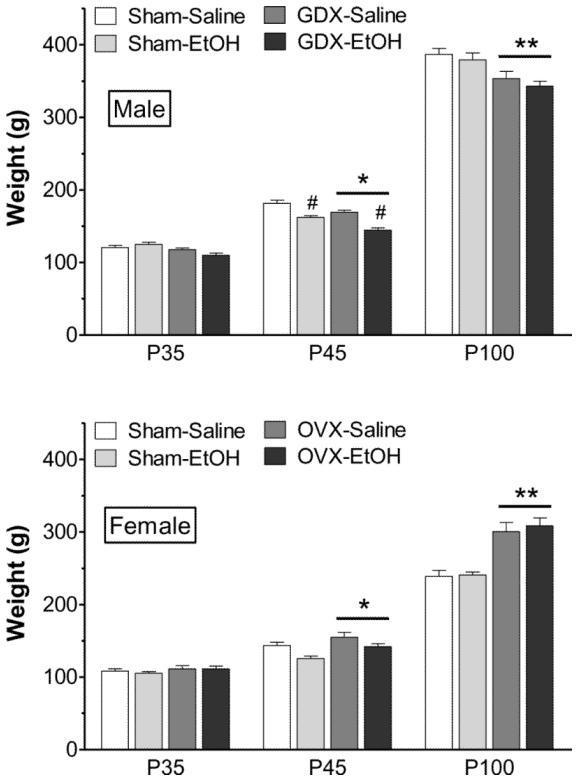

Figure 2.

Body weight of males (top) and females (bottom) before (P35) and after (P45) binge-like ethanol exposure, and also in adulthood (P100). The groups (n = 11–13 each) are sham operated, saline-exposed (Sham-Saline), sham operated, ethanol-exposed (Sham-EtOH), gonadectomy (males) or ovariectomy (females), saline-exposed (GDX-Saline or OVX-Saline), and GDX or OVX, ethanol-exposed (GDX-EtOH or OVX-EtOH). * $p < 0.05$, ** $p < 0.001$, compared to sham operated rats of same age, collapsed across exposure type; $^{\#}p < 0.001$, compared to saline-exposed rats of same age.

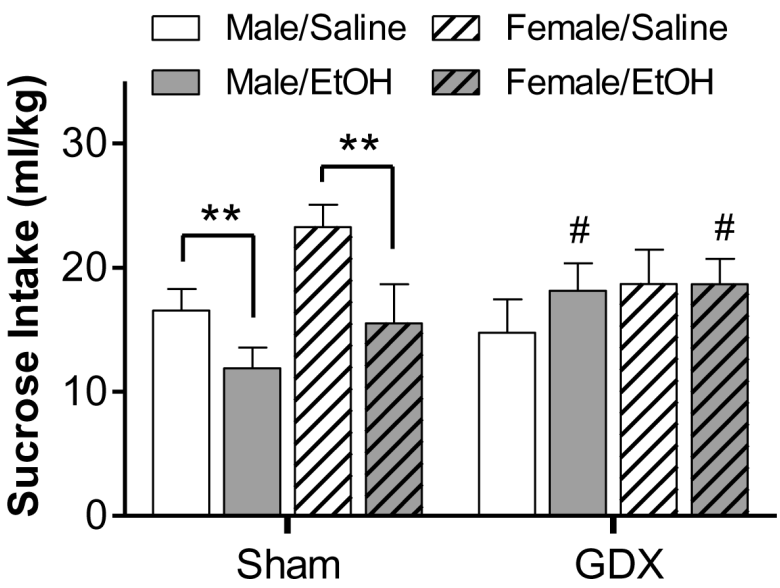

Figure 3.

Sucrose consumption during the initial phase of the sucrose-fading procedure. Shown is the intake of 20% sucrose during a 30-min access period when rats had *ad libitum* access to food and water in their homecage. ** $p < 0.01$ compared to same sex ethanol-exposed shams; $^{\#}p < 0.05$, compared to same sex, ethanol-exposed sham rats

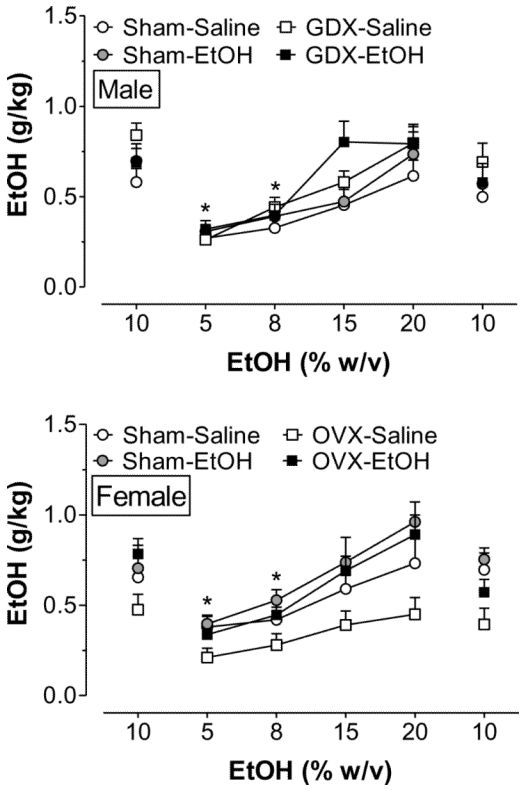

Figure 4.

Concentration response curves for ethanol consumption in adult male (top) and female (bottom) rats. Initially, rats were given access to 10 % ethanol during 10 daily 30 min drinking sessions. When intake was stable at 10%, different ethanol concentrations (5–20 % w/v) were presented in random order for 5 consecutive days each. Finally, rats were given 5 more days of access to 10% ethanol following 10 days of alcohol deprivation. Data are presented as mean ± SEM, averaged across the final 3 days that each concentration was presented. Group labels are defined as in Fig. 3. * $p < 0.05$, compared to intake during the first presentation of 10% ethanol.

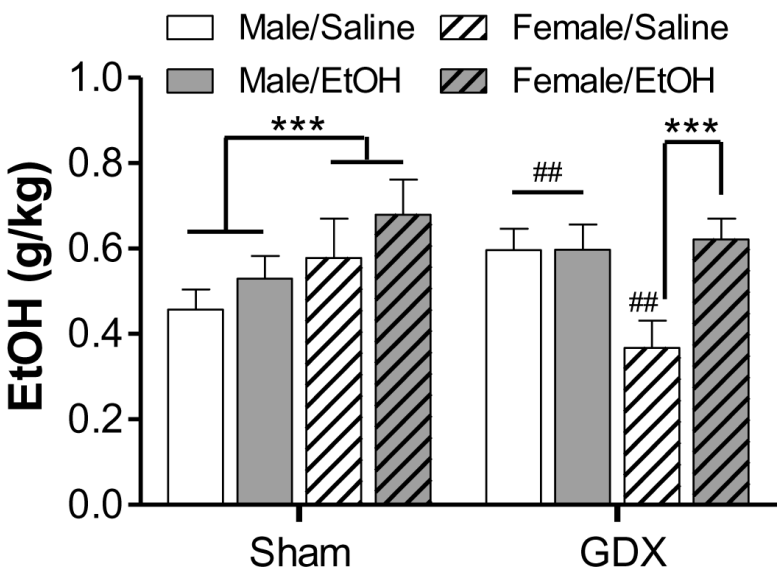

Figure 5.

Drinking behavior collapsed across ethanol concentration (5–20% w/v). The average intake during the last 3 days each concentration was used for this analysis. *** $p < 0.001$; $^{\#\#}p < 0.01$, compared to same-sex, sham-operated controls (collapsed across treatment)