Published in final edited form as:

Behav Brain Res. 2013 March 1; 240: 69–75. doi:10.1016/j.bbr.2012.11.020.

Differences in sensitivity to ethanol-induced conditioned taste aversions emerge after pre- or post-pubertal gonadectomy in male and female rats

Melissa Morales, M.S. and Linda P. Spear, Ph.D.

Center for Development and Behavioral Neuroscience, Department of Psychology, Binghamton University, Binghamton, New York 13902-6000

Abstract

We have previously demonstrated that gonadectomy either prior to (early) or after (late) puberty elevated ethanol consumption in males to levels similar to intact adult females—effects that were attenuated by testosterone replacement. To assess whether alterations in the aversive effects of ethanol might contribute to gonadectomy-associated increases in ethanol intake in males, the present study examined the impact of gonadectomy on conditioned taste aversions (CTA) to ethanol in male and female Sprague-Dawley rats. Animals were gonadectomized, received sham surgery (SH) or non-manipulated (NM) on postnatal (P) day 23 (early) or 67 (late) and tested for CTA to ethanol in adulthood. Water-deprived rats were given 1 hr access every-other-day to 10% sucrose followed by an injection of ethanol (0, 1 g/kg) for 5 test sessions. Test data were analyzed to determine the first day significant aversions emerged in each ethanol group (i.e., sucrose intakes significantly less than their saline-injected counterparts). Early gonadectomized males acquired the CTA more rapidly than did early SH and NM males (day 1 vs 3 and 4 respectively), whereas a gonadectomy-associated enhancement in ethanol CTA was not evident in late males. Among females, gonadectomy had little impact on ethanol-induced CTA, with females in all groups showing an aversion by the first or second day, regardless of surgery age. These data suggest that previously observed elevations in ethanol intake induced by either pre- or post-pubertal gonadectomy in males are not related simply to gonadectomy-induced alterations in the aversive effects of ethanol indexed via CTA.

Keywords

Conditioned taste aversion; ethanol sensitivity; gonadal hormones; sex differences; puberty; rat

1. Introduction

Alcohol is one of the more commonly used drugs of potential abuse, with approximately half of the U.S. population (i.e., 50.9%) aged 18 and older being regular drinkers in 2010 [1]. Unfortunately, adults are not the only age group to consider when examining alcohol consumption patterns. For example, the 2012 Monitoring the Future Study demonstrated that

© 2012 Elsevier B.V. All rights reserved.

Address correspondence to: Melissa Morales, Department of Psychology, Binghamton University PO Box 6000, Binghamton, New York 13902-6000, mmorale2@binghamton.edu, Phone: (607) 777-6418, Fax: (607) 777-6418.

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

13%, 27%, and 40% of 8th, 10th, and 12th graders, respectively, consumed at least one alcoholic drink in the month prior to the survey [2]. Indeed, when adolescents drink, they consume more per drinking episode than do adults [3, 4]. While the detriments of adolescent drinking are multifaceted, there may be an evolutionarily conserved biological component, with higher alcohol consumption rates during adolescence than in adulthood also frequently observed in the rodent literature [5, 6]. Elevated levels of alcohol consumption during adolescence is particularly troubling given that heavy ethanol exposure at this time has been reported to disrupt frontal cortical and hippocampal development in rodents (see [7, 8] for reviews) and to be associated with disrupting neurocognitive functioning in humans (see [9] for review). Thus, it is important to understand why this developmental transition from immaturity to maturity is associated with increased alcohol use relative to other ages.

Because of ethical issues associated with administration of alcohol to underage youth, substantial research has utilized animal models to examine adolescent-typical alcohol intake and sensitivities and their contributors. Use of even simple (e.g., rodent) animal models of adolescence is supported by evidence that many of the hormonal, neural, and behavioral changes characteristic of this developmental transition has been relatively highly conserved across species (see [10, 11]). Preclinical studies using rodent models have demonstrated differences in sensitivity to the rewarding and aversive effects of ethanol in adolescents and adults, providing some insight into the possible reasons for why this developmental period is associated with elevated ethanol intake. For example, adolescents may consume greater quantities of ethanol than adults in part because they are more sensitive to the positive effects of ethanol, indicated by the facilitation of social behavior seen at low doses of ethanol [12], while being less sensitive to the negative/aversive consequences of ethanol that emerge at more moderate ethanol doses and that may normally serve to constrain intake levels (e.g., conditioned taste aversion) [13, 14].

Age differences in ethanol intake are influenced by sex. In adulthood, female rodents generally consume greater amounts of alcohol (g/kg basis) than their male counterparts [5, 14]; such sex differences in ethanol intake patterns are less clear during adolescence, if evident at all [14–16]. Although the directionality of sex differences in ethanol intake in adults is opposite in humans (in terms of number of drinks consumed) than is seen in rodent studies, a similar ontogenetic pattern for the emergence of sex differences in intake is apparent. That is, the drinking levels of males and females are similar up until the age of 17, with sex differences in intake emerging during late adolescence [17]. Among the factors that could contribute to the appearance of more marked sex differences in ethanol intake later in adolescence and into adulthood could be increases in pubertal (e.g., gonadal) hormones (see [18] for review). Consistent with this possibility, boys and girls who mature early sexually have been shown to demonstrate higher levels of alcohol use [19, 20]. Individual differences in general maturational rate, however, may influence not only pubertal timing but timing of non-pubertal-related neurobehavioral developmental processes as well, complicating interpretation of such data.

To explore specific consequences of gonadal hormone-dependent pubertal maturation, pre-clinical studies have examined the emergence of adult-typical sex-dependent behaviors in gonadectomized rodents. This approach has yielded evidence for pubertally-dependent hormone effects on a number of sex-typical reproductive and non-reproductive (e.g., aggression, anxiety) behaviors in adulthood, suggesting an “organizational” role for gonadal hormones at puberty on the neural systems underlying these behaviors (see [21] for review). This approach has also been used to examine the role of pubertal gonadal hormones in mediating sex-related differences in ethanol intake and sensitivity that emerge after adolescence/post-pubertally [22–24]. Our laboratory has recently shown that while ovariectomy either pre-pubertally or in adulthood had little impact on ethanol consumption

in female rats, males gonadectomized either pre- or post-pubertally demonstrated patterns of ethanol intake in adulthood similar to females [22]. Furthermore, following testosterone replacement, ethanol intake levels of gonadectomized adult males were reduced to levels similar to those seen in sham-operated males [23]. Given that similar intake-enhancing effects were seen after pre- as well as post-pubertal castration, with these effects attenuated by testosterone replacement prior to testing in adulthood, these data support an “activational” rather than a pubertally-related “organizational” role for gonadal hormones on ethanol intake in males.

To assess whether gonadectomy-related alterations in ethanol intake in males may be related to alterations in the aversive effects of ethanol, the present study examined the impact of gonadectomy on conditioned taste aversions (CTA) to ethanol in male and female Sprague-Dawley rats. CTA has been used frequently to index the aversive (or dysphoric) properties of drugs by pairing a novel taste stimulus (conditioned stimulus) with the drug (unconditioned stimulus) (see [25] for review). It has been shown that ethanol intake is negatively correlated with CTA [26]—i.e., ethanol’s aversive properties may curb ethanol intake. Therefore, if the elevated ethanol consumption previously observed in gonadectomized males [22–24] was related to a decrease in ethanol-related dysphoria, then ethanol-induced CTA should be less pronounced in gonadectomized relative to sham-operated males. Given that previous results from our laboratory demonstrated that pre-pubertal gonadectomy blocked the normal developmental decline in ethanol intake evident in males in adulthood while not impacting ethanol intake during adolescence [22], the current study focused on CTA to ethanol in animals tested in adulthood following either pre- or post-pubertal gonadectomy.

2. Materials and Methods

2.1 Subjects

A total of 240 male and female Sprague-Dawley rats derived from our breeding facility were used in the current experiments. On postnatal day (P) 1, all litters were culled to 8–10 pups with a sex ratio of six males and four females kept whenever possible. Pups were weaned on P21, pair housed with same-sex littermates, and given ad libitum access to food (Purina Lab chow, Lowell, MA) and water in a temperature-controlled vivarium with a 14:10 hr light-dark cycle (lights on at 0700 hr). At all times animals were treated in accordance with guidelines for animal care established by the National Institutes of Health under protocols approved by the Binghamton University Institutional Animal Care and Use Committee.

2.2 Experimental Design

Ten subjects were placed into each of the 24 experimental groups defined by the 2 age of surgery (early, late) X 2 sex X 2 ethanol dose (0, 1 g/kg) X 3 hormonal status (gonadectomy [GX], sham-operated [SH], non-manipulated [NM]) factorial, with no more than one animal from a litter being placed into any given experimental group.

2.3 Surgery

Animals were anesthetized using isoflurane (3.5% initially) and maintained at surgical levels of anesthesia throughout the surgery via nose cone supplementation (3% repeated as necessary). For castration in males, each testis was removed, a suture made in each tunic and in the inguinal ring (to prevent possible herniation), and the incision closed with Vetbond tissue adhesive (3M, St. Paul, MN). For ovariectomies, an incision was made on the dorsal side of the animal, caudal to the last rib and through the skin perpendicular to the midline. On each side of this incision, an opening was made in the muscle wall via blunt dissection, with the oviduct on each side sutured proximal to the ovary, the ovary excised, and the

muscle wall sutured. For SH surgeries, animals were anesthetized and an incision was made; however, the reproductive tissue was not manipulated nor the gonads removed. At the time of surgery and then again in the afternoon, animals received a subcutaneous injection of the anti-inflammatory agent, ketofen (5 mg/ kg); this two-injection procedure was repeated the following day. Following surgery, animals were given a subcutaneous injection of saline and were returned to their home cages, with a wire-mesh divider used to separate each housing pair for a recovery period of approximately 72 hours. On the fourth day, the divider was removed, and subjects were left undisturbed until testing in adulthood (P74).

2.4 Procedure

Experimental subjects were re-housed on P21 (early: pre-pubertal) or P65 (late: post-pubertal) with a same-age non-littermate of the same sex. Two days later, animals received either GX or SH surgery, or were left alone (NM). Beginning on P74 (between the hours of 10am and 1pm), and continuing every other day throughout the procedure, each pair of animals was water deprived 50%. To calculate water deprivation, water intake for each pair of animals over the previous 24-hr period was measured and half of this amount was provided in a 100-ml bottle. Twenty-four hours after the onset of the water deprivation period, each conditioning session occurred. At the onset of each session, animals were weighed and each housing pair was separated in their home-cage with a wire-mesh divider 15 min prior to the 1 hr conditioning/test session. The wire mesh divider allowed for measurement of individual consumption without the stress of isolate housing, and has been used previously in our laboratory [13]. At the onset of conditioning, each animal was provided with one bottle containing 10% sucrose in tap water. Immediately following the 1-hr access period, the bottle was removed and the animal was injected interperitoneally with either 0 or 1 g/kg ethanol (12.6% v/v solution in 0.9% saline). Each pair of animals housed together received the same drug challenge and remained separated with the wire-mesh divider for an additional 15 min to avoid association of any possible immediate negative effects of the injection with their housing partner. After 15 min, the mesh dividers were removed and each pair of animals was provided with ad libitum access to a fresh bottle of water which was pre-weighed for determination of ad libitum water intake to be used in calculation of water deprivation the following day. This alternating two day procedure of 24 hr of 50% water deprivation followed by a conditioning/test session and 24 hr of ad libitum water was repeated for a total of 5 test days. Thus, after the first conditioning day, each conditioning/test day served as both a test of conditioning from prior days as well as an additional conditioning trial, thereby allowing monitoring of the development of a CTA across days (modified from [27]). Immediately following bottle removal on the final conditioning/test day, animals were sacrificed via rapid decapitation and trunk blood was collected for assay of testosterone, estradiol, progesterone, and corticosterone.

2.5 Hormone assays

Plasma collected from whole blood was stored and maintained at -80°C until time of assay. Testosterone, estradiol (and progesterone) levels were used to confirm removal of the gonadal tissue in males and females, respectively, whereas corticosterone levels were used as a biomarker of stress. For analysis of testosterone, estradiol, and progesterone, plasma samples were thawed and assessed via radioimmunoassay (RIA) using a ^{125}I double antibody kit from MP Biomedicals (Solon, OH), with specificities of 100% for each hormone assayed. Testosterone assay sensitivity was 0.03 ng / ml, with inter- and intra-assay coefficients of variation of 10.1 and 6.6%, respectively. Estradiol assay sensitivity was 7.2 pg / ml, with inter-assay coefficients of 9% and intra-assay coefficients of 7.3%. Progesterone assay sensitivity was 0.11 ng/ml, with inter-assay coefficients of 7.9% and intra-assay coefficients of 5.9%. Samples and standards for each hormone assay were run in duplicate, using a Packard Cobra II Autogamma Counter (PerkinElmer, Boston, MA), with

disintegrations per minute averaged against a standard curve. Plasma CORT levels were determined by RIA using competitive binding tritium-based kits obtained from MP Biomedicals, Inc. with 100% specificity for rat CORT. The sensitivity of the competitive binding assay was 25 pg/tube, with inter-assay coefficients of 10.4% and intra-assay coefficients of 6.1%. Standards and samples were assayed in duplicate, with disintegrations per minute averaged against a standard curve plotted with each assay and input into GraphPad Prism 5.0 software for calculation of concentration in each sample.

2.6 Data Analyses

Prior to all analyses, data were checked for outliers and any value greater or less than 2 standard deviations from the mean was removed. Due to this, 22 intake data points (1%) met this criteria and were replaced with the average intake from the surrounding days for that subject. Additionally, 1 animal was removed prior to analysis due to low baseline intake (i.e., intake <10 ml).

Baseline intake (ml/kg), body weight (g), and hormone levels were analyzed using factorial ANOVAs (i.e., 2 age of surgery X 2 sex X 3 hormonal status). Test day intake (in ml/kg) was analyzed across the 5 test days via repeated measures ANOVAs. Fisher's test was used to explore significant interactions among the groups of interest.

3. Results

3.1 Body weight

The factorial ANOVA of body weight (g) yielded an age of surgery X hormonal status X sex interaction [$F(2, 204) = 25.19, p < 0.00001$]. Fisher's test demonstrated that in females, early GX resulted in significantly higher body weights relative to all other groups (Figure 1, left). In males, an opposite pattern emerged, with early GX resulting in significantly lower body weight relative to early SH and NM males. While no differences emerged between the late females, late NM males weighed significantly more than late GX and SH males (Figure 1, right).

3.2 Sucrose consumption on baseline day

The analysis of baseline sucrose consumption (ml/kg) demonstrated a main effect of age of surgery [$F(1, 222) = 26.82, p < 0.00001$], with late manipulated animals, regardless of sex or surgery condition, consuming significantly less sucrose on baseline day relative to early manipulated animals from the same surgery condition. Additionally, a hormonal status X sex interaction revealed that, in males, GX animals consumed more sucrose than SH and NM males regardless of age of surgery (Figure 2).

3.3 Sucrose consumption on test days

The repeated measures ANOVA for sucrose consumption (ml/kg) across the five test days revealed a 5-way interaction of day X age of surgery X hormonal status X dose X sex [$F(8, 840) = 2.34, p < 0.05$]. There was little impact of hormonal status or age of surgery on sucrose consumption in females: all groups conditioned with ethanol demonstrated and maintained lower intake by the first or second day (Figure 3a). Among males, early GX males conditioned with ethanol consumed significantly less sucrose on all test days relative to saline-injected counterparts, whereas early SH and NM animals only expressed lower sucrose consumption beginning on test days 3 and 4, respectively (Figure 3b, top). An opposite pattern was apparent in late manipulated animals. Late ethanol-treated GX males demonstrated lower sucrose intake compared to saline-treated males starting on test day 3 whereas late SH and NM males consumed significantly less sucrose beginning on days 2 and 1, respectively (Figure 3b, bottom).

3.4 Hormone levels

In females, analysis of estradiol levels revealed a main effect of hormonal status [$F(2,108) = 8.87, p < 0.001$]; regardless of age of surgery, GX females had significantly albeit modestly lower levels of estradiol than SH females, with no difference observed between NM and SH females. In males, the ANOVA for testosterone levels revealed a main effect of hormonal status [$F(2,107) = 57.12, p < 0.00001$], indicating that testosterone levels were significantly lower in GX animals than SH animals, regardless of age of surgery. Interestingly, SH males had significantly higher levels of testosterone relative to NM males, regardless of SH surgery age (see Table 1 for all hormone levels).

Analysis of progesterone revealed an age of surgery X hormonal status X sex interaction [$F(2,213) = 3.23, p < 0.05$]. Early GX females had significantly lower progesterone levels than early SH females, with no difference observed between the early SH and NM females. Among late manipulated females, GX females again had lower levels of progesterone than late SH's, with late SH females having higher levels than late NM. No differences in progesterone were evident across groups in males. Both early and late SH and NM females had significantly higher progesterone levels than their male counterparts.

The corticosterone (CORT) data violated homogeneity of variance and was subsequently transformed via log transformation. The factorial ANOVA demonstrated a main effect of sex [$F(1,219) = 77.60, p < 0.0001$] as well as a hormonal status X sex interaction [$F(2,219) = 6.95, p < 0.001$]. As anticipated, females in all groups had higher levels of CORT compared to comparably treated males. There was also a trend for GX females to have lower CORT levels than SH and NM females, regardless of age of surgery. In males, an opposite pattern emerged: GX males had significantly higher CORT levels relative to SH and NM males.

4. Discussion

The current experiment explored the possibility that alterations in the aversive properties of ethanol (as indexed via CTA) may contribute to the elevated ethanol consumption observed in gonadectomized relative to sham-operated males seen previously in our laboratory [22, 23]. Our results showed that, in males, removal of the testes prior to puberty (i.e., early GX) resulted in animals that showed and maintained an aversion to ethanol after a single ethanol exposure (i.e., day 1). This was different from the effect observed in early SH and NM males, which showed an attenuated aversion to the effects of ethanol, as evidenced by an emergence of a CTA on day 3–4. Post-pubertal removal of the testes (i.e., late GX) resulted in a generally opposite in male rats, with late GX males developing an aversion to ethanol by the third day relative to SH and NM animals that started showing an aversion on days 2 and 2, respectively. In females, ovariectomy had little effect on ethanol-induced CTA. Both early and late GX and SH females exhibited aversions to ethanol by the first or second test day which continued throughout the experimental procedure.

Over the past decades there have been a number of reports of sex differences in CTA in adult rodents [28–31]. Males have been reported to acquire a CTA faster with lithium chloride (LiCl) [30] and drugs of abuse (e.g., ethanol [31]) than females. The faster acquisition of a CTA and slower extinction seen in males has been reported to be hormone dependent [28, 30]. For example, the proportion of males that acquired a LiCl CTA after GX in adulthood was found to be significantly smaller than SH males, an effect reversed by testosterone replacement. The effects in females were less clear in this study, although somewhat more GX females acquired a CTA than did SH females, suggesting a possible role for ovarian hormones as well [30]. The results from our male data do not support the notion that the presence of testosterone increases CTA in males, given that both late GX and SH males acquired a CTA by the second and third test days. In fact, the data from our early

males suggest an opposite effect, with early GX males showing a more rapid expression of ethanol CTA relative to early SH males (day 1 vs day 3, respectively). The inconsistencies between studies may reflect procedural differences (e.g., history of injection, water restriction schedule), strains used, or unconditioned stimulus (e.g., ethanol, LiCl, cocaine). Thus, overall the data are still mixed as to whether males and females develop CTAs differently [14, 32, 33] and what role gonadal hormones play in mediating the aversive properties of ethanol and other drugs of abuse.

In the current study, little impact of GX at either age was seen in females, a finding similar to the limited impact of GX on CTA in females reported by Chambers and colleagues [30]. This lack of impact of GX on ethanol CTA in females is reminiscent of prior research in rodents examining the role of gonadectomy on ethanol intake where little impact of ovariectomy was found on ethanol consumption [22, 34–36]. It is possible that testing intact females (i.e., SH and NM) across varying stages of estrous could have increased variability in our data, making it more difficult to detect clear effects in females. While it has been shown that estrous cycle does impact drug intake in females [37], it also has been shown to have no effect on total ethanol intake in females, changing only the microstructure of drinking bouts [38]. Nevertheless, it is possible that estrous cycle may alter the aversive properties of ethanol in females in a cycle-dependent manner.

Another possibility for the absence of GX effects in females relative to males in the present study may be related to sex differences in the magnitude of surgery-related decreases in gonadal hormones. Although the estradiol levels in early and late GX females were significantly lower than their SH counterparts, the relative decline in estrogen levels was small (~10%)—findings not unexpected given multiple other sources of estrogen (e.g., adrenals, liver, fat, muscle) [39, 40]. Unfortunately, most studies examining GX-related effects on ethanol intake fail to report hormone levels following gonadectomy (see [22] for an exception); thus, it is difficult to say definitively whether residual estradiol levels could have contributed to the general absence in effects of GX on CTA seen in females. While it was not possible to confirm complete removal of the ovaries with estrogen, it is unlikely that much residual ovarian tissue was left in the females given that progesterone levels were significantly and robustly lower in GX than SH females (i.e., ~91% reduction) after surgery at either age. This was expected given progesterone's role in female reproductive function, with the ovary being the key site for production and synthesis of progesterone [41]. Progesterone levels in late SH females differed significantly from NM females, with late SH females having higher levels relative to their NM counterparts, an effect possibly attributable to progesterone's close involvement with stress and anxiety [42].

While the body weight and progesterone data provide clear evidence that the ovaries were appropriately removed in GX females, the estradiol levels we observed both in intact and gonadectomized females (see Table 1) are high relative to those reported by others [43–45]. The differences in estradiol levels may be due to the particular assay used. A recent study [46] investigated estradiol levels in female Sprague-Dawley rats across various stages of the estrous cycle using three popular RIA assay kits: Diagnostic Products Corporation (DPC), Diagnostic Systems Labs (DSL), and MP Biomedical (MPB) (the kit used in the current study). Estradiol levels obtained in intact female rats using these three assays ranged from 6–63, 15–34, and ~114–169, pg/ml, respectively. The authors were unsure as to why the MPB assay yielded significantly higher estradiol levels; however, their levels closely resemble what we observed after using this assay kit, and may have contributed to the surprisingly high levels of estrogen that were observed here.

Although gonadectomy had little impact on CTA, some effects on weight gain were observed in females. For example, reminiscent of previous work from our laboratory [22],

early (i.e., pre-pubertal) ovariectomy resulted in females that weighed significantly more than early SH-operated females, with no differences observed between hormonal status in late-manipulated females, perhaps because of the shorter post-surgery-to-test interval following late than early surgery. These results are consistent with the rodent literature demonstrating weight gain in ovariectomized female rats [47–49]. The hyperphagia and subsequent weight gain that results from ovariectomy has been shown to be reversible with estradiol treatment, suggesting a role for this hormone in regulating food intake [47, 50, 51].

An opposite pattern emerged following pre-pubertal gonadectomy in males. Castration prior to puberty resulted in males that weighed significantly less at the beginning of the experimental procedure in adulthood than did SH-operated males—findings consistent with previous data from our laboratory [22]. While there was no difference between the early SH and NM males, late SH surgery resulted in animals weighing significantly less than late NM. While this effect could possibly reflect an effect of surgical stress, it should be noted that this was not seen in the females or in previous data in males [22]. Effects of late SH were also not evident in terms of corticosterone levels, with similar levels seen in SH and NM males from both surgery ages, again, data that do not support a stress explanation. In contrast, however, males GX at either age showed elevated levels of corticosterone relative to both SH and NM control males—a likely partial feminization of corticosterone levels due to the absence of testosterone [52].

Sucrose consumption is commonly used as a marker of anhedonia with suppressed intake of the sweetened solution presumed to reflect a hedonic deficit typically observed in rodents following a stressor ([53–55], although see [56, 57]). Interestingly, the apparent hedonic value of sucrose was suppressed by late manipulation in both males and females: regardless of hormonal status, rats of either sex that were manipulated post-pubertally (i.e., late GX, SH, or NM) consumed significantly less sucrose at baseline than pre-pubertal-manipulated animals. These data suggest that, perhaps, the manipulation-to-test interval may not have been sufficiently long in late-manipulated relative to early-manipulated animals (7 days vs. >50 days, respectively), to recover from the initial experimental perturbations, and hence they may have been more stressed at time of testing. However, the corticosterone levels between early- and late-manipulated animals were not different, which does not correspond with data showing both higher corticosterone levels and less sucrose consumption in stressed mice relative to their non-stressed counterparts [58]. Other factors may be involved which altered the hedonic value of sucrose between the early and the late manipulated groups.

Baseline sucrose intake also differed between GX and SH-operated males, regardless of age of surgery. Males who were gonadectomized at either age consumed a greater amount of sucrose than their SH counterparts—a difference not observed between GX and SH females. Prior data on sweet taste preference following gonadectomy in male and female rats are mixed, with some reporting that saccharin preference is lower in GX females relative to intact females, with a trend for GX males to have a greater saccharin preference than intact males [59]. In another study, glucose preference in GX females changed as a function of glucose concentration (i.e., decreased intake observed with a 12% solution but increased intake of a 5% solution) relative to pre-GX intake, whereas no GX effects were observed in males [60]. Procedural differences (e.g., sweetener used, concentration) may account for the diversity of findings across studies.

5. Conclusions

Taken together, these data suggest that the increased ethanol consumption following gonadectomy in males is not attributable to an attenuation in the aversive properties of

ethanol. That is, males given early gonadectomy exhibited a stronger CTA to ethanol while late gonadectomized males exhibited an opposite pattern, whereas ethanol intake was similarly elevated after GX at either age among males in prior work in our laboratory [22, 23]. An alternative possibility is that the elevations in ethanol intake following GX in males may reflect a gonadectomy-associated enhancement in sensitivity to the positive (e.g., socially enhancing) effects of ethanol, a possibility currently being investigated in our laboratory.

Acknowledgments

The authors would like to thank Lynda Ruffini for performing the gonadectomy and sham surgeries, as well as her assistance in running the experiment.

Funding: This research was supported by NIAAA grant R01 AA017355 to LPS.

References

- Schiller JS, Lucas JW, Ward BW, Peregoy JA. Summary health statistics for U.S. adults: National Health Interview Survey, 2010. *Vital Health Stat* 10. 2012;1–207. [PubMed: 22834228]
- Johnston, LD.; O'Malley, PM.; Bachman, JG.; Schulenberg, JE. Monitoring the Future national results on adolescent drug use: Overview of key findings, 2011. Ann Arbor: Institute for Social Research, The University of Michigan; 2012.
- Administration SAaMHS. NSDUH Series H-44. Rockville, MD: Substance Abuse and Mental Health Services Administration; 2012. Results from the 2011 National Survey on Drug Use and Health: Summary of National Findings. HHS Publication No (SMA) 12–4713
- Chen K, Kandel DB. The natural history of drug use from adolescence to the mid-thirties in a general population sample. *Am J Public Health*. 1995; 85:41–47. [PubMed: 7832260]
- Doremus TL, Brunell SC, Rajendran P, Spear LP. Factors influencing elevated ethanol consumption in adolescent relative to adult rats. *Alcohol Clin Exp Res*. 2005; 29:1796–1808. [PubMed: 16269909]
- Vetter CS, Doremus-Fitzwater TL, Spear LP. Time course of elevated ethanol intake in adolescent relative to adult rats under continuous, voluntary-access conditions. *Alcohol Clin Exp Res*. 2007; 31:1159–1168. [PubMed: 17511750]
- Crews F, He J, Hodge C. Adolescent cortical development: a critical period of vulnerability for addiction. *Pharmacol Biochem Behav*. 2007; 86:189–199. [PubMed: 17222895]
- Guerri C, Pascual M. Mechanisms involved in the neurotoxic, cognitive, and neurobehavioral effects of alcohol consumption during adolescence. *Alcohol*. 2010; 44:15–26. [PubMed: 20113871]
- Bava S, Tapert SF. Adolescent brain development and the risk for alcohol and other drug problems. *Neuropsychol Rev*. 2010; 20:398–413. [PubMed: 20953990]
- Spear LP. The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev*. 2000; 24:417–463. [PubMed: 10817843]
- Spear LP. Adolescent neurobehavioral characteristics, alcohol sensitivities, and intake: Setting the stage for alcohol use disorders? *Child Dev Perspect*. 2011; 5:231–238. [PubMed: 22328900]
- Varlinskaya EI, Spear LP. Acute effects of ethanol on social behavior of adolescent and adult rats: role of familiarity of the test situation. *Alcohol Clin Exp Res*. 2002; 26:1502–1511. [PubMed: 12394283]
- Anderson RI, Varlinskaya EI, Spear LP. Ethanol-induced conditioned taste aversion in male sprague-dawley rats: impact of age and stress. *Alcohol Clin Exp Res*. 2010; 34:2106–2115. [PubMed: 20860618]
- Vetter-O'Hagen C, Varlinskaya E, Spear L. Sex differences in ethanol intake and sensitivity to aversive effects during adolescence and adulthood. *Alcohol Alcohol*. 2009; 44:547–554. [PubMed: 19767625]

15. Lancaster FE, Brown TD, Coker KL, Elliott JA, Wren SB. Sex differences in alcohol preference and drinking patterns emerge during the early postpubertal period. *Alcohol Clin Exp Res*. 1996; 20:1043–1049. [PubMed: 8892526]
16. Truxell EM, Molina JC, Spear NE. Ethanol intake in the juvenile, adolescent, and adult rat: effects of age and prior exposure to ethanol. *Alcohol Clin Exp Res*. 2007; 31:755–765. [PubMed: 17386073]
17. Substance Abuse and Mental Health Services Administration OoAS. Rockville, MD: 2007. The NSDUH Report: Gender Differences in Alcohol Use and Alcohol Dependence or Abuse: 2004 and 2005.
18. Witt ED. Puberty, hormones, and sex differences in alcohol abuse and dependence. *Neurotoxicol Teratol*. 2007; 29:81–95. [PubMed: 17174531]
19. Patton GC, McMorris BJ, Toumbourou JW, Hemphill SA, Donath S, Catalano RF. Puberty and the onset of substance use and abuse. *Pediatrics*. 2004; 114:e300–e306. [PubMed: 15342890]
20. Tschann JM, Adler NE, Irwin CE Jr, Millstein SG, Turner RA, Kegeles SM. Initiation of substance use in early adolescence: the roles of pubertal timing and emotional distress. *Health Psychol*. 1994; 13:326–333. [PubMed: 7957011]
21. Sisk CL, Zehr JL. Pubertal hormones organize the adolescent brain and behavior. *Front Neuroendocrinol*. 2005; 26:163–174. [PubMed: 16309736]
22. Vetter-O'Hagen CS, Spear LP. The effects of gonadectomy on age- and sex-typical patterns of ethanol consumption in Sprague-Dawley rats. *Alcohol Clin Exp Res*. 2011; 35:2039–2049. [PubMed: 21651581]
23. Vetter-O'Hagen CS, Sanders KW, Spear LP. Evidence for suppressant effects of testosterone on sex-typical ethanol intake in male Sprague-Dawley rats. *Behav Brain Res*. 2011; 224:403–407. [PubMed: 21726585]
24. Sherrill LK, Koss WA, Foreman ES, Gulley JM. The effects of pre-pubertal gonadectomy and binge-like ethanol exposure during adolescence on ethanol drinking in adult male and female rats. *Behav Brain Res*. 2011; 216:569–575. [PubMed: 20816899]
25. Davis CM, Riley AL. Conditioned taste aversion learning: implications for animal models of drug abuse. *Ann N Y Acad Sci*. 2010; 1187:247–275. [PubMed: 20201857]
26. Green AS, Grahame NJ. Ethanol drinking in rodents: is free-choice drinking related to the reinforcing effects of ethanol? *Alcohol*. 2008; 42:1–11. [PubMed: 18164576]
27. Risinger FO, Cunningham CL. Genetic differences in ethanol-induced conditioned taste aversion after ethanol preexposure. *Alcohol*. 1995; 12:535–539. [PubMed: 8590615]
28. Chambers KC. Progesterone, estradiol, testosterone and dihydrotestosterone: effects on rate of extinction of a conditioned taste aversion in rats. *Physiol Behav*. 1980; 24:1061–1065. [PubMed: 7413784]
29. Chambers KC, Sengstake CB. Sexually dimorphic extinction of a conditioned taste aversion in rats. *Anim Learn Behav*. 1976; 4:181–185. [PubMed: 964443]
30. Chambers KC, Sengstake CB, Yoder RL, Thornton JE. Sexually dimorphic acquisition of a conditioned taste aversion in rats: effects of gonadectomy, testosterone replacement and water deprivation. *Physiol Behav*. 1981; 27:83–88. [PubMed: 6267627]
31. Sherrill LK, Berthold C, Koss WA, Juraska JM, Gulley JM. Sex differences in the effects of ethanol pre-exposure during adolescence on ethanol-induced conditioned taste aversion in adult rats. *Behav Brain Res*. 2011; 225:104–109. [PubMed: 21767576]
32. Randall-Thompson JF, Riley AL. Morphine-induced conditioned taste aversions: assessment of sexual dimorphism. *Pharmacol Biochem Behav*. 2003; 76:373–381. [PubMed: 14592690]
33. Busse GD, Freeman KB, Riley AL. The interaction of sex and route of drug administration in cocaine-induced conditioned taste aversions. *Pharmacol Biochem Behav*. 2005; 81:814–820. [PubMed: 16005058]
34. Almeida OF, 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; 101:2677–2685. [PubMed: 9637701]
35. Cailhol S, Mormede P. Sex and strain differences in ethanol drinking: effects of gonadectomy. *Alcohol Clin Exp Res*. 2001; 25:594–599. [PubMed: 11329501]

36. Hilakivi-Clarke L. Role of estradiol in alcohol intake and alcohol-related behaviors. *J Stud Alcohol*. 1996; 57:162–170. [PubMed: 8683965]
37. Fattore L, Altea S, Fratta W. Sex differences in drug addiction: a review of animal and human studies. *Womens Health (Lond Engl)*. 2008; 4:51–65. [PubMed: 19072451]
38. Ford MM, Eldridge JC, Samson HH. Microanalysis of ethanol self-administration: estrous cycle phase-related changes in consumption patterns. *Alcohol Clin Exp Res*. 2002; 26:635–643. [PubMed: 12045471]
39. Nelson LR, Bulun SE. Estrogen production and action. *J Am Acad Dermatol*. 2001; 45:S116–S124. [PubMed: 11511861]
40. Takahashi K. On the extra-ovarian origin of estrogen. *Tohoku J Exp Med*. 1961; 75:207–222. [PubMed: 13919052]
41. Graham JD, Clarke CL. Physiological action of progesterone in target tissues. *Endocr Rev*. 1997; 18:502–519. [PubMed: 9267762]
42. Lovick TA. Estrous cycle and stress: influence of progesterone on the female brain. *Braz J Med Biol Res*. 2012; 45:314–320. [PubMed: 22450372]
43. Dubal DB, Wise PM. Neuroprotective effects of estradiol in middle-aged female rats. *Endocrinology*. 2001; 142:43–48. [PubMed: 11145565]
44. Meli R, Pacilio M, Raso GM, Esposito E, Coppola A, Nasti A, et al. Estrogen and raloxifene modulate leptin and its receptor in hypothalamus and adipose tissue from ovariectomized rats. *Endocrinology*. 2004; 145:3115–3121. [PubMed: 15059958]
45. Kuba T, Wu HB, Nazarian A, Festa ED, Barr GA, Jenab S, et al. Estradiol and progesterone differentially regulate formalin-induced nociception in ovariectomized female rats. *Hormones and behavior*. 2006; 49:441–449. [PubMed: 16257405]
46. Strom JO, Theodorsson A, Theodorsson E. Substantial discrepancies in 17beta-oestradiol concentrations obtained with three different commercial direct radioimmunoassay kits in rat sera. *Scandinavian journal of clinical and laboratory investigation*. 2008; 68:806–813. [PubMed: 18622802]
47. Węgorzewska IN, Walters K, Weiser MJ, Cruthirds DF, Ewell E, Larco DO, et al. Postovariectomy weight gain in female rats is reversed by estrogen receptor alpha agonist, propylpyrazoletriol. *Am J Obstet Gynecol*. 2008; 199:67, e1–e5. [PubMed: 18241818]
48. Chen Y, Heiman ML. Increased weight gain after ovariectomy is not a consequence of leptin resistance. *Am J Physiol Endocrinol Metab*. 2001; 280:E315–E322. [PubMed: 11158936]
49. Jiang JM, Sacco SM, Ward WE. Ovariectomy-induced hyperphagia does not modulate bone mineral density or bone strength in rats. *J Nutr*. 2008; 138:2106–2110. [PubMed: 18936205]
50. Eckel LA, Geary N. Estradiol treatment increases feeding-induced c-Fos expression in the brains of ovariectomized rats. *Am J Physiol Regul Integr Comp Physiol*. 2001; 281:R738–R746. [PubMed: 11506987]
51. Geary N, Asarian L. Estradiol increases glucagon's satiating potency in ovariectomized rats. *Am J Physiol Regul Integr Comp Physiol*. 2001; 281:R1290–R1294. [PubMed: 11557638]
52. Seale JV, Wood SA, Atkinson HC, Harbuz MS, Lightman SL. Gonadal steroid replacement reverses gonadectomy-induced changes in the corticosterone pulse profile and stress-induced hypothalamic-pituitary-adrenal axis activity of male and female rats. *J Neuroendocrinol*. 2004; 16:989–998. [PubMed: 15667454]
53. Strekalova T, Spanagel R, Bartsch D, Henn FA, Gass P. Stress-induced anhedonia in mice is associated with deficits in forced swimming and exploration. *Neuropsychopharmacology*. 2004; 29:2007–2017. [PubMed: 15266352]
54. Christianson JP, Paul ED, Irani M, Thompson BM, Kubala KH, Yirmiya R, et al. The role of prior stressor controllability and the dorsal raphe nucleus in sucrose preference and social exploration. *Behav Brain Res*. 2008; 193:87–93. [PubMed: 18554730]
55. Orsetti M, Canonico PL, Dellarole A, Colella L, Di Brisco F, Ghi P. Quetiapine prevents anhedonia induced by acute or chronic stress. *Neuropsychopharmacology*. 2007; 32:1783–1790. [PubMed: 17213846]
56. Forbes NF, Stewart CA, Matthews K, Reid IC. Chronic mild stress and sucrose consumption: validity as a model of depression. *Physiol Behav*. 1996; 60:1481–1484. [PubMed: 8946494]

57. Matthews K, Forbes N, Reid IC. Sucrose consumption as an hedonic measure following chronic unpredictable mild stress. *Physiol Behav.* 1995; 57:241–248. [PubMed: 7716198]
58. Wann BP, Audet MC, Gibb J, Anisman H. Anhedonia and altered cardiac atrial natriuretic peptide following chronic stressor and endotoxin treatment in mice. *Psychoneuroendocrinology.* 2010; 35:233–240. [PubMed: 19604644]
59. Zucker I. Hormonal determinants of sex differences in saccharin preference, food intake and body weight. *Physiology & Behavior.* 1969; 4:595–602.
60. Gabric D, Soljagic M. Effect of gonadectomy on taste preference for glucose solutions in rats. *Physiol Behav.* 1975; 15:145–148. [PubMed: 1187849]

Highlights

- Pre-pubertal GX resulted in an enhanced EtOH CTA relative to SH and NM males
- Post-pubertal GX did not alter EtOH sensitivity compared to intact males
- In females, GX resulted in little impact on EtOH sensitivity regardless of surgery age
- Late manipulation altered the hedonic value of sucrose in males and females

Figure 1. Body weight (g) of female (left) and male (right) gonadectomized (GX), sham-operated (SH), or NM rats. Data are presented as mean $\pm$ SEM. \$ indicates a significant difference between early GX and SH animals of the same sex. @ indicates a significant difference between late SH and NM males. $p < 0.05$.

Figure 2. Sucrose intake (ml/kg) on baseline day is presented for females (left) and males (right). # denotes a significant difference between early and late animals across all surgery conditions. * denotes a significant difference between GX and SH males, regardless of age of surgery.

a

b

Figure 3.

Sucrose intake (ml/kg) across the 5 test days is shown for females (Figure 3a) and males (Figure 3b) for early (top panel) and late (bottom panel) surgery conditions. * represents a significant difference between ethanol- and saline-treated animals of a given sex and age of surgery group for that test day.

Table 1

Hormone levels (mean SEM) in females (left) and males (right)

Females				Males			
Hormonal Status	Estradiol (pg/ml)	Progesterone (ng/ml)	Corticosterone (ng/ml)	Hormonal Status	Testosterone (ng/ml)	Progesterone (ng/ml)	Corticosterone (ng/ml)
Early				Early			
GX	112.99±3.93^a	4.65±0.35 ^a	183.75±32.50 ^c	GX	0.00±0.00 ^a	3.78±0.31	110.95±13.69^{ab}
SH	122.96±3.00	50.82±5.38 ^c	207.16±36.71 ^c	SH	2.71±0.25 ^b	3.51±0.17	41.56±6.96
NM	142.13±6.93	53.74±5.86 ^c	254.10±42.10 ^c	NM	2.20±0.37	3.29±0.24	43.67±12.81
Late				Late			
GX	117.88±2.38^a	5.40±0.36 ^a	140.79±24.76 ^c	GX	0.00±0.00 ^a	4.01±0.41	81.00±13.57^{ab}
SH	132.35±4.28	58.25±8.95 ^{bc}	214.21±33.39 ^c	SH	2.23±0.35 ^b	3.63±0.24	67.85±14.09
NM	133.82±8.48	35.44±4.67 ^c	189.20±35.46 ^c	NM	1.46±0.18	3.35±0.33	50.70±12.18

^a GX animals differed significantly from same-sex SH counterparts^b SH animals differed significantly from same-sex NM counterparts.^c Sex difference for the corresponding hormonal status of the same age.**Bold** denotes an effect collapsed across age ($p < 0.05$).