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FEMALE RATS DISPLAY ENHANCED REWARDING EFFECTS OF ETHANOL THAT ARE HORMONE DEPENDENT

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Abstract

Background—Ethanol abuse is a major health and economic concern, particularly for females who appear to be more sensitive to the rewarding effects of ethanol. This study compared sex differences to the rewarding and aversive effects of ethanol using place-conditioning procedures in rats.

Methods—Separate groups of adult (male, female, ovariectomized [OVX] female) and adolescent (male and female) rats received ethanol (0, 0.5, 1.0, 2.0 or 2.5 g/kg; ip) and were confined to their initially non-preferred side of our conditioning apparatus for 30 minutes. On alternate days, they received saline and were confined to the other side. Following 5 drug pairings, the rats were re-tested for preference behavior. Separate cohorts of the same groups of rats were injected with a similar dose range of ethanol and blood ethanol levels (BELs) were compared 30 minutes later.

Results—Ethanol produced rewarding or aversive effects in a dose-dependent manner. An intermediate dose of ethanol (1.0 g/kg) produced rewarding effects in adult female but not male or OVX female rats, suggesting that ovarian hormones facilitate the rewarding effects of ethanol. Similarly, this intermediate dose of ethanol produced rewarding effects in adolescent female but not male rats. The highest dose of ethanol (2.5 g/kg) produced aversive effects that were similar across all adult groups. However, the aversive effects of ethanol were lower in adolescents than adults, suggesting that adolescents are less sensitive to the aversive effects of ethanol. The aversive effects of ethanol did not vary across the estrous cycle in intact adult females. There were also no group differences in BELs, suggesting that our results are not related to ethanol metabolism.

Conclusion—Our results in rats suggest that human females may be more vulnerable to ethanol abuse due to enhanced rewarding effects of this drug that are mediated by the presence of ovarian hormones.

Keywords

alcohol; reward; aversion; adolescence; sex differences

INTRODUCTION

Epidemiological evidence suggests that women are more vulnerable to ethanol abuse. For example, women become intoxicated at faster rates and become ethanol-dependent more readily than men (Greenfield 2002; Mancinelli et al. 2009). Women are also more likely to develop ethanol-related health problems, such as liver failure, heart attack, cancer and

osteoporosis as compared to men (Epstein et al. 2007). In addition, women display more neurotoxicity and brain damage following ethanol exposure relative to men (Ceylan-Isik et al. 2010; Hommer et al. 1996; Prendergast 2004). However, women may not be more vulnerable to all aspects of ethanol abuse, as they initiate ethanol use later in life, consume less amounts of ethanol per occasion, and are more likely to remain abstinent after quitting relative to men (York and Welte 1994). Ethanol possesses both appetitive (rewarding) and aversive effects that play a role in ethanol use. The contribution of the rewarding and aversive effects of ethanol to the abuse liability of this drug in women and men has not been well characterized. Thus, this study used a pre-clinical animal model that compares rewarding and aversive effects of ethanol in female and male rats of different ages.

Pre-clinical studies in rodents suggest that females experience greater rewarding effects of ethanol as compared to males. For example, females display greater voluntary intake of ethanol versus water in two-bottle choice procedures in rats (Almeida et al. 1998; Cailhol and Mormede 2001; Lancaster and Spiegel 1992; Sluyter et al. 2000; Vetter O'Hagan et al. 2009) and mice (Middaugh et al. 1999a; Tambour et al. 2008). Consistent with this, females display greater ethanol intake than males in operant procedures in rats (Blanchard et al. 1993) and mice (Middaugh et al. 1999b). Female rats that were genetically bred to prefer ethanol (P rats) also consume more ethanol than male P rats during peri-adolescence and adulthood under both continuous (Bell et al. 2006) and binge-like (Bell et al. 2011) access conditions. Taken together, these reports suggest that the rewarding effects of ethanol are enhanced in female versus male rodents. However, we also acknowledge reports showing that female mice display similar conditioned place preference (CPP) produced by ethanol as compared to males (Itzhak et al. 2009; Nocjar et al. 1999), and female rats consume less ethanol at higher concentrations as compared to males (van Haaren and Anderson 1994).

Previous studies have also compared sex differences to the rewarding effects of ethanol in rodents from different stages of development. For example, ethanol-induced CPP was observed in female mice that were tested during the early and late phases of adolescence, whereas males only displayed CPP during the early phase of adolescence (Roger-Sanchez et al. 2012). Adolescent female mice also consume more ethanol compared to their male counterparts (Tambour et al. 2008). In contrast to the latter findings; however, Vetter O'Hagan et al. (2009) showed that adolescent female rats drink less ethanol as compared to adolescent males. In addition, Lancaster et al. (1996) showed no differences in ethanol drinking in adolescent female and male rats. The inconsistent findings with regard to sex differences in adolescent mice and rats may be related to species-specific differences in the mechanisms that modulate the behavioral effects of ethanol (see Green and Grahame 2008).

One important aspect to consider when studying adult female rodents is hormone fluctuations that occur across the 4-day estrous cycle (proestrus, estrus, metestrus and diestrus). A study comparing ethanol self-administration across the estrous cycle observed a reduction in ethanol intake during estrus (Roberts et al. 1998). However, the latter finding was only observed in female rats that were pharmacologically synchronized, but not in females that were allowed to cycle freely. One possible explanation is that estrous cycle synchronization may have elicited behavioral changes, such as arousal and/or hyperactivity that could have interfered with operant behavior given that the synchronized females entered estrus simultaneously. Ford et al. (2002a) also showed that ethanol intake was similar across estrous in freely cycling female rats. Although the behavioral effects of ethanol may not vary across estrous, we recognize that the neurochemical effects of ethanol may fluctuate across estrous. This is based on a microdialysis study showing that ethanol produces the largest increase in dopamine levels in the medial prefrontal cortex during estrus when estrogen levels peak (Dazzi et al. 2007).

The influence of hormones on the rewarding effects of ethanol has also been examined using ovariectomy (OVX) procedures. For example, OVX female rats display lower levels of ethanol intake in two-bottle choice procedures as compared to intact females (Almeida et al. 1998; Cailhol and Mormede 2001). Consistent with this, baseline ethanol intake was significantly reduced following OVX procedures (Ford et al. 2002b). A follow up study established that the latter effect was reversed with estradiol replacement (Ford et al. 2004). Further validating the importance of ovarian hormones, ethanol-induced increases in dopamine levels in the medial prefrontal cortex are blunted in OVX females as compared to intact females or OVX females that received estrogen pre-treatment (Dazzi et al. 2007). These studies highlight the importance of ovarian hormones in mediating the putative rewarding effects of ethanol in females.

The goal of this study was to examine the influence of sex, age, and ovarian hormones on the rewarding and aversive effects of ethanol. The place-conditioning paradigm assesses the motivational properties of a drug by means of Pavlovian conditioning. Ethanol is administered in a distinct environment and after several pairings the environmental cues becomes associated with the effects of the ethanol, thereby acquiring incentive-motivational properties (Tzschentke 2007). Following conditioning, the environmental cues elicit either approach (CPP) or avoidance (CPA) depending on whether rewarding or aversive effects were elicited by different doses of ethanol during conditioning. The place-conditioning paradigm involves sub-chronic passive administration of a fixed dose of ethanol, which does not mimic the voluntary intake pattern observed in humans. Procedures involving ethanol drinking assess the appetitive properties of ethanol that are presumed to be evidence of high ethanol reinforcement (Green and Grahame 2008). Thus, place-conditioning studies assess the association between the rewarding or aversive effects of ethanol with external environmental cues, whereas drinking studies assess the appetitive properties of ethanol. Given our goal of comparing sex differences to the rewarding and aversive effects of ethanol, this study compared place conditioning produced by various doses of ethanol in separate groups of adult male, female, OVX female and adolescent male and female rats. This study also examined whether the magnitude of aversive effects produced by ethanol varied across the 4-day estrous cycle. Lastly, blood ethanol levels (BELs) were compared across our experimental groups in order to assess the influence of ethanol metabolism in the results.

METHODS

Animals

Adult (PND 60-75) and adolescent (PND 28-45) male and female Wistar rats were handled for 3 days prior to experimentation. The animals were housed in a humidity- and temperature-controlled (22°C) vivarium at the Psychology Department of the University of Texas at El Paso (UTEP). All rats were bred from a stock of outbred Wistar rats from Harlan, Inc. Animals were weaned on PND 21 and group housed with same-sex litter mates 2-3 per cage. Rats had *ad libitum* access to standard rodent chow and water, except during conditioning and preference testing. Rats were kept on a reverse light/dark cycle with lights on at 8:00 pm, such that all testing procedures were conducted in the dark phase. All procedures and experimental protocols were approved by the UTEP Institutional Animal Care and Use Committee (IACUC) and were conducted in adherence to the NIH Guidelines for the Care and Use of Laboratory Animals.

Study 1: Place conditioning

Apparatus—Our conditioning apparatus consisted of 2 rectangular stainless steel chambers (76 × 24 × 30 cm) with 1-way mirrors on the front walls to allow for behavioral

observations. Each chamber was divided into 2 distinct compartments of equal proportions that were separated by a removable solid stainless steel partition and elevated above different types of bedding. One compartment had pine bedding beneath a smooth Plexiglas® floor with small holes. The other compartment had green-tinted chlorophyll bedding beneath a metal bar floor. The rats were tested in a dark room under red light with both compartments equally illuminated with white light during the conditioning and testing procedures. It is recognized that the white light may have produced anxiety behavior in the apparatus. However, these conditions were kept constant for all experimental groups, and therefore, do not likely explain any observed group differences. While white light during the dark-cycle can induce some phase/circadian-shifting, which may in turn influence daily fluctuations in hormone levels, these possibilities will need to be addressed with multiple control conditions in future studies. Background noise was provided during conditioning and testing by an industrial air filter system that minimized outside disturbances (approximately 50 dB; Honeywell Inc.). Female and male rats were run in separate cohorts to avoid the influence of pheromones on our results. Following conditioning, each apparatus was thoroughly cleaned with Wex-cide detergent, 70% ethanol, and then water.

Experimental groups—Study 1 compared place conditioning produced by various doses of ethanol (0, 0.5, 1.0, 2.0 or 2.5 g/kg, ip) in male (n=11-20 per group) and female (n=11-30 per group) and OVX female (n=8-14 per group) adult rats. Adult rats received the initial preference test on PND 60, conditioning began on PND 67, and the final preference test was conducted on PND 77. In order to compare sex differences in adolescent rats, place conditioning produced by the same doses of ethanol was also compared in adolescent male (n=6-10 per group) and female (n=7-9 per group) rats. Adolescent rats received the initial preference test on PND 28, conditioning began on PND 34, and the final preference test was conducted on PND 45.

Conditioning procedures—This study employed a biased conditioning procedure consisting of 3 phases: an initial preference test, 5 conditioning trials, and a final preference test. A biased procedure was used because it produces reliable and repeatable results in our laboratory. In our assessment of the literature, more than half of the studies examining ethanol CPP in rats use a biased procedure. Our conditioning procedures are similar to previous reports examining ethanol CPP in rats (for a review see Fidler et al. 2004).

A pre-test was first conducted in order to determine the rats' initially non-preferred side. Rats were later conditioned with ethanol in their initially non-preferred side, as defined by the side where they spent less than 50% of their time during the pre-test. However, rats that spent less than 35% of their time on the non-preferred side during the pre-test were eliminated from the study. This criterion was employed because it is difficult to detect a shift in time spent in a compartment where an animal has a strong initial bias prior to conditioning. This is particularly important for studies employing place-conditioning procedures with drugs that possess weak reinforcing properties such as ethanol in rats.

During preference testing, all rats were placed in the middle of the apparatus. For consistency, all rats were placed into the apparatus facing the same chamber (chlorophyll bedding with metal bar floor). They were then allowed to shuttle freely between the 2 compartments for 15 minutes, a time of testing that has been widely used in our laboratory and that of others (see Fidler et al. 2004; Torres et al. 2008 and 2009). A short test period was used to avoid habituation to the test chamber, which facilitates the expression of preference behavior that is based on previous drug-environment associations. Rats were considered to have entered a compartment if the 2 front paws were placed on the floor of that compartment. An observer that was blind to the animals' treatment condition scored the time spent in each compartment.

The first conditioning day was conducted 6 days after the initial pre-test. The rationale for the delay period was to attempt to minimize the effects latent inhibition on conditioning. Although there is no specific rationale for using a 6-day period, the delay was intended to separate the exposure to the drug-paired compartment in the absence of ethanol during the pre-test. This is important given that the purpose of conditioning is to pair the effects of ethanol with the same environmental cues that the rats were exposed to during the pre-test. Furthermore, a recent report showed that pre-exposure to the CPP apparatus before conditioning eliminated CPP produced by ethanol in rats (1.0 g/kg; Morales et al. 2012). Despite this, it is recognized that many laboratories examining ethanol CPP employ more than one pre-test, since a single baseline trial may be an index of exploratory behavior. To address this issue, we conducted a study comparing the magnitude of CPP produced by ethanol (1.0 g/kg) in female rats (n=16-18 per group) given 1 or 2 pre-tests. The data revealed that there were no significant differences in the magnitude of CPP in rats that received 1 (128 ± 26) versus 2 (103 ± 16) pre-tests. These values approximate those depicted in Figure 1. These data also revealed that the amount of activity was similar on both sides of the conditioning apparatus, suggesting that activity levels do not predict the magnitude of CPP produced by ethanol (data not shown).

During conditioning, a solid partition separated the chambers so that the rats could be confined to one side of the conditioning apparatus. The rats were injected with saline or ethanol and were placed immediately into their initially non-preferred side for 30 minutes. The saline injections were for the control rats. Separate groups of rats received different doses of ethanol. On alternate days, the rats received saline and were confined to their initially preferred side for 30 minutes. This 2-day procedure was repeated over 10 consecutive days. The order of drug treatment was counterbalanced such that half of the rats from each treatment group received ethanol on the first day of conditioning and the other half received ethanol on the second day of conditioning. Control groups received saline on both days of conditioning. The ethanol solutions were prepared from 95% ethanol diluted in 0.9% sterile saline.

The day after the last conditioning session, a final preference test was conducted. All animals were allowed to shuttle freely between the two distinct compartments of our conditioning apparatus for 15 minutes.

Estrous determination—After the final preference test, adult female rats received vaginal lavage procedures to determine the phase of the estrous cycle they were in during the final preference test (i.e., proestrus, estrus, metestrus or diestrus). Adolescent females were not subjected to the lavage procedures due to the undifferentiated nature of their epithelium cells. Adolescent females of the age range in the present study are not yet regularly cycling (Forbes and Dahl 2010; Sisk and Foster 2004). A sterile and disposable plastic pipette was filled with 0.9% saline and was used to collect epithelial cells. Epithelial cells were then transferred to a labeled glass microscope slide. Microscope slides were fixed with methylene blue stain (Sigma Inc.) and viewed under a light microscope at 40 X to examine the shape of the cells and determine the phase of the estrous cycle by the following criteria: proestrus=presence of round nucleated epithelium cells, estrus=presence of cornified un-nucleated epithelium cells, metestrus=presence of leukocytes, and diestrus=limited presence of epithelium cell and leukocytes. A greater number of intact females were included in the 2.5 dose group so that fluctuations across the estrous cycle could be studied at a high ethanol dose. A high dose of ethanol was used to assess behavioral fluctuations across estrous because this dose produced the most robust behavioral effects, and this increased the likelihood of detecting behavioral differences across estrous.

OVX procedures—The OVX procedure was conducted in young female rats (PND 40-45) that were sedated using isoflurane gas. An incision 5-8 mm long was made at a point about 1 cm anterior to the knee and 2 cm ventral to the spinal cord. The tissue was separated through the inner layers of connective tissue and then the ovaries were isolated and ligated. The ovaries were then cut away from the oviduct distal to the ligature. The ends of the oviduct were then placed back inside the body cavity followed by suturing the connective tissue and skin. Animals then received flunixin (2.5 g/kg, sc) and were allowed to recover for 15 days prior to testing. After the final preference test, an additional 4 days of vaginal lavage procedures verified that our OVX procedure prevented estrous cycling.

Study 2: Ethanol plasma levels

Study 2 compared BELs in separate groups of naïve rats. Adult (male, intact female, and OVX female) and adolescent (male and female) rats ($n=4-10$ per group) received a similar dose range of ethanol (0.5, 1.0, or 2.0 g/kg) that was used in Study 1. Thirty minutes later, blood samples were collected from tail veins and centrifuged for 15 minutes at $5000 \times g$ at 4°C . Plasma was then stored at -80°C until analyzed using an Analox AM1 instrument for determining BELs in rodent plasma (Analox Instruments, Lunenburg, MA).

Statistical Analysis

Difference scores were used as the dependent measure, which reflect a shift in preference from the pre- to post-test for each animal. The difference scores were calculated as the amount of time spent in the initially non-preferred compartment after conditioning minus before conditioning, such that positive values reflect rewarding effects whereas negative values reflect the aversive effects of ethanol. CPP was operationally defined as a significant increase in the difference score obtained from ethanol-treated rats as compared to control rats that received saline during conditioning. In contrast, CPA was defined as a significant decrease in the difference score obtained from ethanol-treated versus control rats.

Our statistical analyses of difference scores (Figure 1) and BELs (Figure 3) included overall ANOVAs with ethanol dose and group (intact adult female, OVX female, adult male, adolescent male and adolescent female) as between subject factors. Our statistical analyses of difference scores across the estrous cycle (Figure 2) included an overall one-way ANOVA across groups of rats that were tested during different phases of estrous. Where appropriate, significant overall effects were followed by individual post-hoc comparisons using Fisher's LSD tests ($p < 0.05$). The comparisons that were made between different groups are denoted with different symbols in the graphs. In Figure 1, the asterisks (*) denote a significant difference from respective saline controls, daggers (†) reflect a difference from respective male counterparts at a particular dose, and number signs (#) reflect a difference from respective adult counterparts at a particular dose. In Figure 3, the asterisks (*) denote a significant difference from the lowest dose of ethanol.

RESULTS

Figure 1 displays the effects of various doses of ethanol (0, 0.5, 1.0, 2.0, or 2.5 g/kg, ip) in adult (male, intact female, and OVX female) and adolescent (male and female) rats. The results revealed a significant interaction of ethanol dose and group [$F(16,270) = 2.17$; $p < 0.05$]. Subsequent post-hoc analyses revealed that intact adult females conditioned with the 1.0 g/kg dose of ethanol displayed a significant positive shift in preference (CPP) as compared to saline controls ($*p < 0.05$), and the magnitude of this effect in females was significantly higher than adult males and OVX females ($\dagger p < 0.05$). CPP was also observed in adolescent females conditioned with the 1.0 g/kg dose of ethanol versus saline controls ($*p < 0.05$), and the magnitude of this effect was significantly higher in adolescent females

versus adolescent males ($\dagger p < 0.05$). A significant negative shift in preference (CPA) was observed in all adult groups that were conditioned with the 2.0 and 2.5 g/kg dose of ethanol as compared to their respective controls ($*p < 0.05$). However, CPA was only observed in adolescent rats that were conditioned with the 2.5 g/kg dose of ethanol versus saline controls ($*p < 0.05$). Furthermore, the degree to which the highest dose of ethanol produced CPA in adolescent male and female rats was lower than their respective adult counterparts ($\#p < 0.05$).

One concern is whether OVX procedures influenced our behavioral outcomes. To address this issue, an additional group of female rats ($n=7-12$) received sham OVX procedures and were conditioned with various doses of ethanol (0, 1.0 and 2.5 g/kg), as described previously (data not shown). The results revealed that there were no differences in the behavioral effects of ethanol across sham and OVX rats [$F(1,87) = .04$, $p = 0.84$]. Namely, similar difference scores were observed in sham OVX (0 = 15.43 ± 19 ; 1.0 g/kg = 132.4 ± 40 ; 2.5 g/kg = -118.3 ± 36) and intact (0 = 28.9 ± 23 ; 1.0 g/kg = 128.16 ± 25 ; 2.5 g/kg = -158.56 ± 22.4) female rats.

Figure 2 displays avoidance behavior in intact female rats conditioned with the 2.5 g/kg dose of ethanol and tested during different phases of the estrous cycle. The results revealed that there were no differences in avoidance behavior produced by ethanol across the various phases of the estrous cycle [$F(3,20) = .96$, $p = 0.42$]. In order to further examine whether preference behavior was altered across estrous, an additional analysis was performed that collapsed the data across all doses of ethanol (data not shown). This analysis allowed us to compare behavioral effects with more rats per estrous condition (estrus $n=11$; diestrus $n=12$; metestrus $n=15$; proestrus $n=17$). The analysis confirmed that there were no significant differences in preference behavior across the estrous cycle [$F(3,51) = .32$; $p > .81$].

Figure 3 displays BELs following acute administration of various doses of ethanol (0.5, 1.0, or 2.0 g/kg, ip) in separate groups of adult (male, intact female, and OVX female) and adolescent (male and female) rats. The results revealed that there was no interaction between ethanol dose and group [$F(8,75) = 0.9$; $p = 0.5$]. However, there was a main effect of ethanol dose [$F(2,75) = 90.4$; $p < 0.001$], suggesting that ethanol produced an increase in BELs in a dose-dependent manner across all groups. Adult rats that received the 1.0 and 2.0 g/kg dose of ethanol displayed an increase in BELs as compared to rats that received the 0.5 g/kg dose of ethanol ($*p < 0.001$). Adolescent rats that received the 2.0 g/kg dose of ethanol also displayed an increase in BELs as compared to rats that received the 0.5 g/kg dose of ethanol ($*p < 0.001$).

One concern is that the BEL data reflect group differences in ethanol pharmacokinetics following the first exposure to ethanol. However, the rats in Study 1 received 5 injections of ethanol during conditioning. Thus, an additional study compared BELs in response to a single injection of ethanol (1.0 g/kg) in naïve rats versus rats that had previously received 5 injections of the same dose of ethanol ($n=5-10$ per group). The results revealed that there were no statistically significant differences in BELs in naïve rats (115.8 ± 5.0 mg/dl) versus animals that were pre-exposed to ethanol (123.6 ± 6.4 mg/dl; $p = 0.4$; data not shown).

DISCUSSION

The main finding of this report is that the rewarding effects of ethanol are enhanced in female rats. To summarize, adult and adolescent female rats displayed enhanced CPP produced by an intermediate dose of ethanol (1.0 g/kg) as compared to males that did not show CPP at any dose of ethanol. The rewarding effects of ethanol appear to be mediated by the presence of ovarian hormones, as OVX females did not display CPP at any dose of

ethanol. Our findings are not likely related to sex differences in ethanol metabolism, as there were no differences in BELs across groups of rats that received different ethanol doses. This lack of group differences is consistent with previous reports showing similar BELs across age (Walker and Ehlers 2009) and sex (Silveri and Spear 2000) shortly after ethanol administration.

Our findings are consistent with previous reports showing enhanced rewarding effects of ethanol in female versus male rodents. For example, adult females voluntarily consume more ethanol as compared to males in two-bottle choice procedures in rats (Cailhol and Mormede 2001; Lancaster et al. 1992; 1996; Sluyter et al. 2000; Vetter O'Hagan et al. 2009) and mice (Middaugh et al. 1999a; Tambour et al. 2008). Adult females also perform more operant responses for ethanol as compared to male rats (Blanchard et al. 1993) and mice (Middaugh et al. 1999b). Taken together with the present findings, these studies suggest that the rewarding effects of ethanol are enhanced in female rodents.

Another major finding of this study is that the rewarding effects of ethanol were absent in OVX females. This finding suggests that the presence of ovarian hormones is important for the expression of ethanol reward in adult females. Consistent with this, OVX female rats display lower levels of ethanol consumption relative to their intact counterparts (Almeida et al. 1998; Cailhol and Mormede 2001; Ford et al. 2002b; 2004). In addition, ethanol produces an increase in dopamine levels in the medial pre-frontal cortex of intact female rats, an effect that is blunted in OVX females (Dazzi et al. 2007). The present study also found that the aversive effects of ethanol were similar in intact and OVX female rats. Taken together, our findings suggest that the presence of ovarian hormones influences the rewarding, but not aversive effects of ethanol.

There are several candidate ovarian hormones that may modulate the present findings. A recent review paper supports the importance of estrogen in modulating enhanced vulnerability to several different drugs of abuse in females (Becker et al. 2012). The role of estrogen in mediating the rewarding effects of ethanol in females is supported by the finding that estrogen replacement re-establishes high levels of ethanol intake in OVX female rats (Ford et al. 2004). Estrogen replacement also normalizes ethanol-induced dopamine release in the pre-frontal cortex of OVX females (Dazzi et al. 2007). Future studies involving replacement procedures are needed to strengthen our conclusion regarding the role of specific hormones, such as estrogen in facilitating the rewarding effects of ethanol in female rats.

The present study also revealed that the aversive effects produced by a high dose of ethanol were similar in adult females that were tested during different phases of the estrous cycle. This finding suggests that hormonal *fluctuations* do not influence the aversive effects of ethanol. This is consistent with a report showing that operant responding for ethanol was similar across the estrous cycle in freely cycling female rats (Roberts et al. 1998). However, the possibility exists that place conditioning and/or operant procedures are not sensitive enough to detect the influence of hormonal fluctuations on the behavioral effects of ethanol. Indeed, Ford et al. (2002a) conducted a microstructural analysis of ethanol intake and found that bout frequency was increased during proestrus relative to all other phases of the estrous cycle. Future studies are needed to examine the influence of hormonal fluctuations across estrous, particularly in rats receiving a dose of ethanol that produces rewarding effects in adult female rats.

The present study also revealed that adolescent females display enhanced rewarding effects of ethanol versus adolescent males. This finding is consistent with the observation that adolescent female mice display CPP produced by ethanol in the early and late phases of

adolescence, whereas males only display CPP produced by ethanol in the early phase of adolescence (Roger-Sanchez et al. 2012). Our findings are also consistent with studies showing that adolescent females consume more ethanol compared to adolescent male rats (Truxell et al. 2007) and mice (Tambour et al. 2008). Researchers employing ethanol-drinking procedures interpret high drinking levels to reflect the strong rewarding effects of ethanol. Our data showing that adolescent rats display reduced aversive effects suggests that young animals may also be consuming high levels of ethanol due to reduced sensitivity to the aversive effects of ethanol. Taken together, these studies suggest that adolescent females display increased sensitivity to the rewarding effects of ethanol as compared to adolescent males. Our finding that adolescent females that are not yet regularly cycling display enhanced ethanol CPP suggests that there may be other (non-ovarian hormone) mechanisms that contribute to the strong rewarding effects of ethanol in adolescent females. These might include cholinergic and/or amino acid systems that have been shown to promote drug use in adolescent rats (Spear 2000; Trauth et al. 2000).

The present study also found that ethanol did not produce CPP in male rats (see Figures 1 and 3). Our lack of preference behavior is in line with previous reports in adult male rats (Blatt and Takahashi 1999; Jones et al. 2009). Although CPP may be difficult to establish with ethanol in naive rats, previous studies have shown that pre-treatment with ethanol before conditioning facilitates this effect in male rats (Beinkowski et al. 1996; Biala and Kotlinska 1998; Ciccocioppo et al. 1999; Gauvin and Holloway 1991; Maldonado-Devincci et al. 2010; Quintanilla and Tampier 2011) and mice (Nocjar et al. 1999). Regarding the lack of CPP in adolescent males, a previous report demonstrated that adolescent male mice did not show ethanol-induced CPP when they were tested at PND 45, which is the same age that the adolescent rats were tested in our study (Roger-Sanchez et al. 2012). However, the latter study reported ethanol-induced CPP in early adolescent mice that were tested at PND 30. Consistent with this, ethanol-induced CPP has been reported in juvenile rats that were tested at PND 25 (Philpot et al. 2003). Future studies focusing on developmental differences to ethanol-induced CPP might include adolescent male and female rats from an earlier stage of development (less than PND 45).

In conclusion, our data suggest that adult and adolescent females display enhanced sensitivity to the rewarding effects of ethanol relative to males. Our findings also suggest that the presence of ovarian hormones facilitate the rewarding effects of ethanol in females. Thus, heightened vulnerability to ethanol abuse in human females may be the result of enhanced sensitivity to the rewarding effects of ethanol. Future work is needed to better understand the influence of ovarian hormones on the neural mechanisms that mediate enhanced vulnerability to ethanol abuse amongst females.

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Figure 1.

Place conditioning produced by various doses of ethanol (0, 0.5, 1.0, 2.0, or 2.5 g/kg, ip) in adult male (n=11-20), adult female (n=8-30), and OVX female (n=7-14), adolescent male (n=6-10), and adolescent female (n=7-9) rats. The data are presented as difference scores ($\pm$ SEM), which reflect time spent in the initially non-preferred side after conditioning minus before conditioning such that values above "0" reflect a positive shift in preference (CPP) and values below "0" represent a negative shift in preference (CPA). Asterisks (*) denote a significant difference from respective saline controls, daggers (†) reflect a difference from respective male counterparts at a particular dose, and number signs (#) reflect a difference from respective adult counterparts at a particular dose ($p < 0.05$).

Figure 2.

Place conditioning produced by ethanol (2.5 g/kg) in adult females that were tested during estrus (n=8), diestrus (n=5), metestrus (n=5), or proestrus (n=6). The data are presented as difference scores ($\pm$ SEM), which reflect time spent in the initially non-preferred side after conditioning minus before conditioning such that values below “0” represent a negative shift in preference (CPA).

Figure 3.

Plasma BELs 30 minutes after administration of various doses of ethanol (0.5, 1.0 or 2.0, g/kg, ip) in adult male (n=5 per dose), adult female (n=4-5 per dose), OVX female (n=4-5 per dose), adolescent male (n=10 per dose), and adolescent female (n=5-6 dose) rats. The asterisks (*) denote a significant increase in BELs relative to the lowest dose of ethanol (0.5 g/kg) collapsed across treatment groups ($p < 0.05$).