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Oral self-administration of EtOH: Sex-Dependent Modulation by Running Wheel Access in C57BL/6J Mice

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Abstract

Background—The effects of stress, including neuroendocrine and behavioral sequelae aimed at maintaining homeostasis, are associated with increased alcohol consumption. Because both stress and drinking are multifactorial, the mechanisms underlying the relationship are difficult to elucidate. We therefore employed an animal model investigating the influence of blocked access to a running wheel on drinking in C57BL/6J (B6) mice.

Methods—In the first experiment, naïve, adult male and female subjects were individually housed for 2 weeks with 24hr access to a running wheel and 12% EtOH in a 2-bottle, free choice paradigm. After determining baseline consumption and preference, experimental subjects had the running wheel placed in a locked position for 6hr, and the EtOH bottle was removed during the first half of this period. Two subsequent experiments, again in adult, naïve B6 mice, examined the influence of locked running wheels on self-administration of 20% EtOH in a limited access paradigm, and blood EtOH concentrations (BECs) were determined.

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Results—In all three studies, using both between and within subject analyses, females showed transient yet reliable increases in alcohol drinking during blocked access to a rotating activity, while drinking in male mice was largely insensitive to this manipulation, though both sexes showed appreciable BECs (>130 mg/dL in females, and 80 mg/dL in males) following a 2hr EtOH access period.

Conclusions—These data add to a burgeoning literature suggesting that the factors contributing to excessive alcohol use differ between males and females, and that females may be especially sensitive to the influence of wheel manipulation. Elucidating the sex-dependent mechanisms mediating differences in alcohol sensitivity and response is critical to understanding the causes of alcoholism and in developing effective treatments and interventions.

Keywords

Stress; Exercise; Addiction; Alcohol; Tension-reduction

Both genetic and environmental factors contribute to the etiology of alcohol abuse and dependence. Exposure to stress is one environmental factor postulated to play a role in the initiation and maintenance of alcohol consumption (Brown et al., 1995; Kushner et al., 2000; Hunt and Zakhari, 1995). According to the tension reduction hypothesis, “stress increases alcohol consumption because alcohol relieves the psychological and physiological consequences of stress, such as tension, anxiety, or physical pain” (Chester et al., 2006). The clinical relationship between stress and drinking is well substantiated, though poorly understood (Bolton et al., 2006; Sinha et al., 2011), and animal studies have yielded inconsistent findings (Correia et al., 2009; Lynch et al., 1999; Boyce-Rustay et al., 2008; Chester et al., 2006). Full understanding of the mechanisms underlying the relationship between stress and alcohol consumption is likely impeded by both the complexity of the stress response and of ethanol’s actions and effects, evidencing the need for appropriate animal models (Amario, 2010; Becker et al., 2011).

In recent years, several researchers have adopted a new approach to this problem by studying relationships between exercise and alcohol consumption (Werme et al., 2002; Ozburn et al., 2008; Ehringer et al., 2009; Pichard et al., 2009). There is mounting evidence that exercise protects against the deleterious effects of chronic stress (Droste et al., 2003; Greenwood and Fleshner, 2008; Onksen et al., 2012). Perhaps by providing an alternative coping strategy regular physical activity has been shown to effectively prevent and treat stress-related disorders including anxiety (Manger and Motta, 2005), depression (Babyak et al., 2000; Knubben et al., 2007), and substance abuse (Zschucke et al., 2012). Furthermore, although alcohol abuse and dependence, anxiety disorders, and major depressive disorder are highly comorbid (cf: Hettema et al., 2003) the shared neural mechanisms involved are not clear. Studying connections between alcohol and exercise may yield new insights into common neural pathways activated by alcohol, exercise, and stress, as well as those underlying the interplay of stress-related disorders.

The wheel running activity of rodents is particularly amenable to studies investigating the biological underpinnings of stress-related disorders and relationships with exercise because wheel running engages brain systems related to reward, mood, and the stress response

(Brene et al., 2007; Greenwood et al., 2011). Voluntary wheel running is an appetitive phenomenon, and rats and mice will lever-press for wheel access (Belke et al., 2005). Rats will also develop conditioned place preference (CPP) to environments associated with wheel running (Lett et al., 2000). Regions of the mesocorticolimbic system that are activated by drugs of abuse are also activated by wheel running (Rhodes et al., 2003; Vargas-Perez et al., 2003). Moreover, long-term repeated voluntary wheel running produces plastic changes in the reward circuitry (Greenwood et al., 2011).

Considerable data also suggest that wheel running has antidepressant effects, including stimulation of hippocampal neurogenesis (Crews et al., 2004; Onksen et al., 2012) and dampening of HPA hormone responses to emotional stressors (Droste et al., 2003). Running may also reduce anxiety-related behaviors in mice (Salam et al., 2009), and wheel running has been shown to increase following exposure to acute stress (footshock; Sibold et al., 2011). Several studies have investigated the effects of wheel running on voluntary alcohol consumption in rodents. When they include both sexes (i.e., Ehringer et al., 2009) it seems that female mice are more likely than males to increase EtOH self-administration during periods when running wheel access or rotations are blocked. It is not clear whether this is because activity displaces drinking as a substitute positive reinforcer or whether it is because blocking access induces an anxiety-like state that is then self-medicated by EtOH. However, the latter hypothesis is supported by clinical reports of gender differences in the risk for and prevalence of stress-related disorders and in line with sex-dependent trajectories for alcohol abuse and dependence (Brady & Randall, 1999; Lynch *et al.*, 2002; Fattore *et al.*, 2008; Zilberman *et al.*, 2003). Overall, women suffer from stress-related psychiatric disorders at about double the rate of men (Kessler et al., 1993; Marcus *et al.*, 2005). One purpose of our experiments was therefore to further explore the role of sex in EtOH self-administration as it relates to running wheel activity.

Several studies have investigated the effects of wheel running on voluntary alcohol consumption. Access to a running wheel decreased EtOH intake in rats bred to accept EtOH (P rats), but did not affect rats bred not to accept EtOH (NP rats; McMillan et al., 1995). However, Crews et al. (2004) found no difference in EtOH consumption between male C57BL/6 mice with wheel access and males without wheel access. Likewise, Ozburn et al. (2008) found no effect of alternating periods of running wheel access on EtOH consumption in female C57BL/6 mice, though these mice chronically consumed EtOH prior to wheel running. Ehringer et al. (2009) also evaluated the effect of running wheel access on established EtOH drinking and found that male and female C57BL/6 mice consumed less alcohol when given access to running wheels, although the effect was less robust for males. All of these studies introduced activity wheels to animals that had first been habituated to a drinking protocol, in some cases for several weeks. Because we hypothesized that disrupting established voluntary activity would lead to changes in drinking, in our studies the running wheels, along with alcohol, were available at the start of the experiment.

Thus we extended this area of inquiry by investigating the influence of running wheel access on the acquisition and maintenance of two-bottle free choice drinking in male and female C57BL/6J mice. In all studies, activity wheels were present in the home cage throughout the experimental period and baseline drinking was concomitant with free running wheel access.

The first experiment evaluated the effect blocking wheel rotations during a three hour period on self- administration of 12% EtOH. Two additional studies examined the effect of alternating wheel access on EtOH intake within and between subjects using limited access to 20% EtOH, again in a two-bottle, free choice paradigm.

Methods

Subjects

Adult naïve male and female C57BL/6J (B6) mice were used in these experiments. Mice were bred in-house using stock purchased from Jackson Laboratories (Bar Harbor, ME) and group housed by sex with 4–5/Plexiglas cage following weaning at 20–21 days. They were maintained in a colony room at 21 ± 2 °C with *ad lib* food and water on a reverse 12:12 light:dark cycle. Food and water were also continuously available throughout the experimental period.

Experimental Procedures

Experiment 1—This experiment employed 28 female mice and 24 male mice. The mice were individually housed in Plexiglas cages with corncob bedding and a low-profile wireless running wheel (Med Associates, St. Albans, VT). Each cage also contained two 25 mL graduated cylinders: one with 12% EtOH in tap water (vol: vol; available through a sipper tube) and the other containing plain tap water. During the experimental period lights were on from 1AM to 1PM in order to accommodate researchers' class schedule, and subjects were habituated to this change and all aspects of the experimental setup for at least 2 weeks before the start of the study.

After the habituation period, oral self-administration was evaluated 4 times per day over 6 days, at 1PM, 4PM, 7PM, and 10PM. Baseline drinking was assessed on days 1 & 2 and used in determining group assignments to ensure equivalent baseline drinking across experimental groups. The two experimental groups were designated "Lock" and "Free" in reference to the status of the running wheel during a 6hr manipulation (1–7 PM) on days 3 & 4 at the start of the dark phase of the cycle. The 2-day test procedure on days 3 & 4 was to record fluid levels at 1PM, put the running wheels in the lock position for the half the animals, and remove the EtOH bottle for all mice (as had occurred during the 2 day baseline period). At 4PM the EtOH bottle was returned for all mice and fluid levels were recorded again. At 7PM the fluid levels were recorded, the wheels for the Lock group were unlocked, and levels were recorded again. Throughout the study, at 10PM the bottles were refilled if necessary and side position was switched to deter the development of side preferences. Empty cages with EtOH and water bottles were used to calculate leakage due to evaporation or inadvertent bumping of the cage rack, and though this was minimal, when necessary these values were subtracted from all other cages to obtain accurate consumption volumes. During the test period, "Free" subjects had continuous access to the running wheels, while "Lock" animals had access to the wheel except during the 6hr manipulation.

Experiment 2—This experiment used 36 male and female mice ($n = 18/\text{sex}$) maintained exactly as in Experiment 1 except that the lights were off from 7AM to 7PM. In this study

the habituation period was shortened to 5 days. During this period, and throughout the experiment, access to 20% EtOH (vol:vol in tap water) was restricted to a 2-hr interval from 10AM to 12PM. After the 5-day “baseline” period, mice were separated into Lock and Free conditions, again to ensure that there were no extant differences between experimental groups. During the subsequent 10 days, from 9AM to 12PM running wheels were put in a “lock” position *every other day* for the Lock group, while the Free group was left undisturbed. “Free” animals had 24hr access to the activity wheels and “Lock” subjects had access for 21hr or 24 hr on alternate days. Thus this design evaluated the effects of blocking running wheel access both between (as in Experiment 1) and within groups.

Experiment 3—Experiment 3 was carried out in exactly the same way as Experiment 2, except that all subjects (n=18, 9 female, 9 male) were subject to the same procedure: 5 days of baseline drinking (2hr, 20%) with running wheel access and then a 10day period of 2hr EtOH access and alternating Locked and Free running wheels. Therefore, unlike subjects in Experiment 2, subjects in the “Lock” condition for this experiment were not exposed to auditory or other cues associated with wheel running on alternate days when they were not able to access rotating wheels, as there was no consistently “Free” control group. Also, on the final day of this experiment, immediately following the 2hr drinking period (at 12PM) blood was sampled from the submandibular vein by a trained experimenter and immediately analyzed for EtOH concentration (AM1 Analyzer, Analox Instruments, Lunenburg, MA).

Data Analysis

Preference for EtOH was assessed as the percentage of fluid consumed from the EtOH-filled bottle, and the dosage was calculated as g of EtOH administered/kg of body weight. Because we were interested in observing the effect of the wheel manipulation on EtOH drinking, we excluded subjects that failed to drink from the bottle containing EtOH at all throughout the baseline period. In Experiment 1, 8 subjects (5 female, 3 male) had preference and dosage scores of “0” during the 2 baseline days prior to the wheel manipulation and were thus excluded; a single female mouse was excluded on this basis in Experiment 2; and none were excluded from Experiment 3. In addition, one statistical outlier (> 2 standard deviations from the group average; a female in the Lock condition) was also excluded from Experiment 1.

Consumption and preference data for each of the time periods (1–4 PM, 4–7 PM, 7–10 PM and 10 PM – 1PM in Experiment 1 and the 2hr period of EtOH access in Experiments 2 & 3 were separately evaluated in a 2 (condition- lock vs. free) x 2 (sex) repeated measure (day) ANOVA using SPSS Statistics 17.0. The change in drinking was evaluated by calculating a within subject difference score between drinking on Locked vs. Free days, and subjecting these data to a one-sample t-test using the null hypothesis that Locked – Free = 0. Simple effects were analyzed from significant interactions using Fisher’s least significant difference (LSD) test. In all cases the criterion for significance was set at $p < 0.05$.

Results

Experiment 1

Compared to baseline consumption, female C57BL/6J mice increased their self-administration of EtOH as a result of having their wheels locked (Figure 1A). Here we compared drinking during each of the test days relative to baseline drinking during the 2 days prior to manipulation by separate 2-factor repeated measure (Sex and Wheel Condition) ANOVAs comparing each test day with the average baseline consumption. There were no differences in baseline drinking patterns within Sex (i.e., assignment to Lock or Free conditions was based on baseline drinking). Evaluating the first test day, females drank more than males overall ($F_{(1,39)} = 9.798, p < 0.01$) but neither Condition ($F_{(1,39)} = 0.005, p > 0.05$) nor its interaction with Sex affected the dosage of EtOH administered ($F_{(1,39)} = 0.350, p > 0.05$). There was a main effect of Day ($F_{(1,39)} = 8.08, p < 0.01$) as well as a Day $\times$ Sex interaction ($F_{(1,39)} = 18.08, p < 0.001$) but no interaction of Day with Wheel Condition nor triple interaction (p 's > 0.05). In the analysis of the second test day, once again females administered higher dosages of EtOH than males ($F_{(1,39)} = 5.623, p < 0.05$) and there was no overall effect of Condition ($F_{(1,39)} = 0.029, p > 0.05$) and no interaction between these variables ($F_{(1,39)} = 1.685, p > 0.05$). Drinking differed across Days ($F_{(1,39)} = 4.695, p < 0.05$) and there was a non-significant trend suggesting that Sex might influence this change ($F_{(1,39)} = 3.175, p = 0.083$) but no interaction between Day and Condition ($F_{(1,39)} = 0.026, p < 0.05$). However, in this analysis there was a triple interaction indicating that the change in drinking across Days was influenced by Condition, but only in one Sex ($F_{(1,39)} = 4.749, p < 0.05$). Post-hoc analysis of simple effects revealed that females in the locked condition consumed more than at baseline ($p < 0.05$).

The data were much the same with respect to preference for the EtOH solution (Figure 1, lower panels). The first day of testing compared to baseline drinking showed that females preferred the EtOH solution more than males ($F_{(1,39)} = 8.506, p < 0.01$), but neither Condition ($F_{(1,39)} = 0.374, p > 0.05$) nor its interaction with Sex influenced preference ($F_{(1,39)} = 0.690, p > 0.05$). There was a main effect of Day ($F_{(1,39)} = 18.09, p < 0.001$) as well as a Day $\times$ Sex interaction ($F_{(1,39)} = 14.54, p < 0.01$) but no significant interaction between Day and Condition ($F_{(1,39)} = 1.493, p > 0.05$) nor triple interaction ($F_{(1,39)} = 0.984, p > 0.05$). With respect to the second test day, there was a tendency (non-significant) for females to have a higher preference than males ($F_{(1,39)} = 3.681, p = 0.062$). There was no overall effect of Condition ($F_{(1,39)} = 1.417, p > 0.05$) nor significant interaction between Sex and Condition ($F_{(1,39)} = 0.880, p > 0.05$). Again, we saw that the preference for EtOH changed across the experimental period (a main effect of Day ($F_{(1,39)} = 16.57, p < 0.001$)). While there was no significant Day $\times$ Sex interaction ($F_{(1,39)} = 1.159, p > 0.05$), there was a Day $\times$ Condition interaction ($F_{(1,39)} = 4.244, p < 0.05$) and again, a triple interaction ($F_{(1,39)} = 4.274, p < 0.05$) reflecting the fact that females increased their preference for the EtOH-filled tube when their running wheels were locked.

Experiment 2

This experiment, employing 2hrs of limited daily access to 20% EtOH in a within-subject design, yielded a similar pattern in which only females increased drinking in response to

locked running wheels. These results, averaged for each of the 3 experimental periods, are summarized in the left hand panels of Figure 2, with g/kg consumption on top (A) and preference below (A'). We calculated a difference score by subtracting average consumption on the 5 Free Days from average consumption on the 5 Lock Days (Figure 2B). Again, only females whose wheels were locked showed a significant difference (from zero) between these two measures. Thus, in terms of g/Kg consumed, the 2×2 (Sex and Condition) ANOVA on difference scores indicated no main effect of Sex ($F_{(1,31)} = 2.254, p > 0.05$), a main effect of Condition ($F_{(1,31)} = 20.071, p < 0.001$) and a Sex $\times$ Condition interaction ($F_{(1,31)} = 10.762, p < 0.01$). Difference in preference for the alcohol filled tube (2B') between Lock and Free Days also indicated no main effect of Sex ($F_{(1,31)} = 2.706, p > 0.05$) but a main effect of Condition ($F_{(1,31)} = 17.027, p < 0.001$). There was not an interaction between Sex $\times$ Condition ($F_{(1,31)} = .378, p > 0.05$).

One sample t-tests conducted independently comparing the average difference score (in Locked groups) of each sex to the null value of zero, showed that both difference scores for females were > 0 : for the difference in g/kg $t_8 = 6.368$ for females, and 0.744 for males; and for preference, both groups differed significantly from 0: $t_8 = 4.380$ for females and 2.944 for males, though this reflected only a 7.8% increase in preference for the EtOH solution (p values for females, but not males, < 0.05).

Experiment 3

Experiment 3, employing 2hrs of limited daily access to 20% EtOH with alternating days of locked and free wheels, provided the third replication of the general finding that female but not male C57BL/6J mice reliably increase their consumption of and preference for EtOH when their access to a running wheel is blocked. Figure 3 summarizes these data in the 18 subjects in this experiment, with g/kg in the top panels, and preference in the bottom panels. A single factor (Sex) repeated measures ANOVA (3 periods including average of Baseline, Lock and Free days) revealed a main effect of Sex on dosage ($F_{(1,16)} = 5.496, p < 0.05$), an effect of Day ($F_{(2,16)} = 4.804, p < 0.05$) and a Sex $\times$ Day interaction ($F_{(2,16)} = 5.692, p < 0.05$). Groups did not differ in terms of EtOH preference: Sex, ($F_{(1,16)} = 2.125, p > 0.05$), Day ($F_{(2,32)} = 1.498, p > 0.05$) and Sex $\times$ Day ($F_{(2,16)} = 1.051, p > 0.05$). On the right panels of Figure 3(B and B') are the difference scores for consumption and preference (respectively). Again, females increased their consumption when the wheels were Locked, but males did not, indicated by a main effect of Sex ($F_{(1,16)} = 5.275, p > 0.05$). One sample t-tests conducted independently comparing the average difference score of each sex to the null value of zero, showed that both difference scores for females were > 0 : for the difference in g/kg $t_8 = 5.607$ for females, and 1.60 for males; and for preference, $t_8 = 2.952$ for females and 0.903 for males (p values for females, but not males, < 0.05).

Immediately following the final 2hr of EtOH access (on the last day of the experiment when all running wheels were in the lock position) blood was sampled and analyzed for EtOH concentration, and these data are shown in Figure 4. Unfortunately, 2 samples were not successfully obtained due to experimenter error (both males) resulting in 7 males and 9 females for this analysis. Though there was a wide range of intakes, both between and within sexes, there were not significant sex differences $F_{(1,17)} = 1.662, p > 0.05$, despite the

fact that overall self administration resulted in pharmacologically relevant blood EtOH concentrations for many subjects: an average of 132.58 ± 28.24 mg/dL in females, and 83.17 ± 25.91 mg/dL in males. The overall correlation between BECs and the dosage of EtOH administered during the two hour period of access was significant (Pearson Correlation = .688, $p < .05$) though this was mostly driven by females where Pearson's r was significant at 0.774 while in males the correlation was weaker ($r = 0.55$, > 0.05).

Discussion

Female C57BL/6J mice reliably increase alcohol drinking in response to having a home cage running wheel switched to a "lock" position, and therefore prevented from rotating. Using different 2-bottle, free choice paradigms employing either 24hr access to 12% EtOH, or 2hr access to 20% EtOH, females who had their running wheels locked responded by transiently increasing their self-administration of EtOH, into a range associated with substantial pharmacological intoxication, while drinking in male C57BL/6J mice was generally insensitive to this manipulation. These findings, obtained using different models of voluntary consumption, provide converging support for the contention that the factors influencing EtOH drinking are at least in part sexually dimorphic.

In contrast to our experiments that afforded access to both wheels and alcohol at the start, previous investigations of voluntary activity and alcohol self-administration established drinking before introducing activity wheels (McMillan et al., 1995; Crews et al., 2004; Ozburn et al., 2008; Prichard et al., 2009) thus examining whether the opportunity for voluntary activity affected stable EtOH drinking. The only one of these studies that included both male and female subjects (C57BL/6 mice, Ehringer et al. 2009) found that both sexes of C57BL/6 mice consumed less alcohol when given access to running wheels, although the effect was less robust for males. In our experiments, drinking in male mice was entirely insensitive to the influence of wheel access. There are at least two potentially important differences between the present studies and those of Ehringer et al.. Again, we introduced the EtOH and activity wheels together at the start of the experiment (in their study subjects had access to wheels before EtOH was introduced), and Ehringer et al., employed an induction period where the concentration of available EtOH (in water) was increased over several days (while ours was constant at either 12% for the study involving 24 hr. access, or 20% in the limited access paradigm). Nonetheless, our studies taken together with those of Ehringer et al. suggest that the alternative reinforcement afforded by the opportunity to exercise may be sexually dimorphic.

In the present study, blocking running wheel access resulted in increased voluntary consumption of EtOH in female, but not male mice. These data suggest that removing an appetitive stimulus (activity wheel access) produced an aversive state that motivated females to increase their consumption of EtOH. It is not currently known how sex modulates the salience of the appetitive wheel stimulus, as only a handful of studies investigated reinforcing effects of wheel running, and most of these only employed male subjects (Lett et al., 2000 & 2001; Belke & Wagner, 2005; Greenwood et al., 2011). There was an early study by Iversen (1993) evaluating reinforcing properties of the wheel in female rats by making access contingent upon lever pressing, though this study didn't include males, thus no

studies in the literature directly compare the sexes. Sex differences in voluntary exercise are well documented, though the pattern of results is complex, and the mechanisms are not at all understood (see, for instance Bowen et al., 2011, 2012 or Lightfoot 2008 for reviews).

Sex differences evident in our study are in line with clinical observations that females are more likely to drink in response to negative emotions (Greenfield et al., 2010) and are more generally sensitive to stress-related drinking (Fattore et al., 2008; Marcus et al., 2005 Hunt and Zakhari, 1995; Noronha et al., 2000). Thus, evidence supports the idea that males and females respond differently to stressors, including EtOH, and that differences in neurobiology mediate this variation (Greenfield et al., 2010; Bangasser and Valentino, 2012). Though as a general concept, the term stress is somewhat ambiguous and far-reaching (McEwen, 2012), the present data suggest that examining EtOH consumption following the removal of an appetitive stimulus may be a useful, though admittedly narrow, model for studies of sex differences in stress reactivity, coping, and voluntary alcohol consumption. Though results from these studies may not generalize to other stressors or conditions this simple model permits us to definitively explore particular factors influencing alcohol drinking; ones that are clearly sex-specific and thus likely to provide a new tool useful for elucidating sex differences in pathology, an important area that remains understudied and therefore poorly understood, despite mounting clinical imperative.

Understanding mechanisms mediating sex differences in alcohol use patterns is critical to understanding the heterogeneous disease. It may be that testosterone buffers the subjective effects of blocking the running wheel, or that estradiol (and its derivatives) renders females more vulnerable (Figueiredo et al., 2007; Larkin et al., 2010). In humans and other animals, females have a stronger HPA axis response to stressors and alcohol than males (Ogilvie and Rivier, 1997; Gallucci et al., 1993). Furthermore, it has been reported that fluctuations in gonadal hormones throughout the ovarian cycle affect HPA activation by EtOH (Rivier, 1993). Larkin et al. (2010) has shown that, in female rodents, stimulation of the HPA axis by EtOH is greatest when levels of estradiol are highest (during the estrous and proestrous stages of the estrous cycle). Bangasser et al. (2010) recently reported evidence for sex differences in stress response signaling at the molecular level by demonstrating that the signaling and trafficking of the receptor for corticotropin-releasing factor (CRF), a key regulator of the HPA axis response to stress, differs between males and females. As a result, CRF-receptive neurons in females are more sensitive to low levels of CRF and less adaptable to high levels of CRF (see Bangasser and Valentino, 2012 for a recent review). Because chronic HPA axis dysregulation is associated with the development of mood disorders (Gold and Chrousos, 2002) and alcohol addiction (Uhart et al., 2006), the sex differences in CRF signaling and trafficking (which differentially affect regulation of the HPA axis) can render females more sensitive to stress and more vulnerable to developing stress-related disorders.

The paucity of basic neuroscience research on females reflects a bias that continues despite ample evidence of sex differences in the physiological and behavioral response to stress and alcohol (Devaud et al., 2003; Fattore et al., 2008). A comprehensive review by Beery and Zucker (2011) across 10 major fields of biomedical research found that neuroscience had the most striking sex bias, with male subjects outnumbering females by at least 5.5 to 1. Neuroscience research on drug abuse is certainly no exception, and the neural mechanisms

underlying sex differences remain largely unknown. Though the NIH mandated inclusion of women in human trials in 1993 there is no analogous initiative for studies employing animal models, and thus the majority of published alcohol research has employed only male subjects, primarily to avoid possibly confounding results due to cycling hormones in females. Although women drink less than men overall, adverse consequences accumulate more rapidly (Wiren et al., 2006; Randall et al., 1999). Given the fact that women represent an increasingly large segment of the drug-dependent population, more basic research is certainly warranted.

These data add to the growing body of research suggesting that the factors influencing propensity to excessive alcohol use differ between males and females (Fattore et al., 2008). There is ample evidence of physiological and behavioral variation in the response to stress and EtOH between the sexes (Bangasser et al., 2010; Rivier, 1993). Because stress and the ability to cope with stressful stimuli are implicated as causal factors in the development of alcoholism (Brown et al., 1995; Bolton et al., 2006), investigation of the mechanisms underlying sex differences in the stress response and the response to alcohol may lead to a better understanding of the sex differences contributing to disease susceptibility (Wiren et al., 2006). Elucidating the sex-dependent mechanisms mediating differences in alcohol sensitivity and response is critical to understanding the causes of alcoholism and in developing effective treatments and interventions.

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Fig. 1.

Preventing rotation of a running wheel (Lock) increased drinking in female B6 mice as measured by the average ($\pm$ SEM) dosage of EtOH self-administered during a 6-hr period of experimental manipulation of running wheel access, beginning 3hr after lights off, during 24-hr, 2-bottle free choice access to 12% EtOH in (A) females and (B) males. The lower panels (A' & B') indicate a sex-dependent increase in preference ($\pm$ SEM) for the EtOH solution in these mice, again with females on the left and males on the right. An asterisk (*) is used to designate within group differences ($p < 0.05$) in drinking compared to baseline, as evident from analysis of simple effects drawn from significant interactions in the 2 (sex) $\times$ 2 (condition) repeated measure ANOVA done separately for each test day. There were 8–11 subjects in each experimental group.

Fig. 2.

Female-specific sensitivity to locked running wheel is evidenced in **Panel A** showing the average ($\pm$ SEM) dosage of EtOH self-administered during the 3 periods of Experiment 2 (Baseline, Lock and Free days). The lower left panels (**A'**) show preference ($\pm$ SEM) for the EtOH solution in at the same time in these mice, again with females on the left and males on the right. An asterisk (*) is used to designate within group differences ($p < 0.05$) in drinking compared to baseline, as evident from analysis of simple effects drawn from significant interactions in the 2 (sex) $\times$ 2 (condition) repeated measure ANOVA done separately for each test day. Difference scores, comparing at the change in preference between Lock and Free days across the experimental groups, are depicted in the right panels for consumption (**B**) and preference (**B'**), and the asterisk (*) reflects changes in drinking that differ significantly from zero. There were 8–9 subjects in each group.

Fig. 3.

In a within subject study only female drinking is sensitive to locked running wheels as evidenced in **Panel A** showing the average ($\pm$ SEM) dosage of EtOH self-administered during the 3 periods of Experiment 3 (Baseline, Lock and Free days). The lower left panels (**A'**) show preference ($\pm$ SEM) for the EtOH solution in at the same time in these mice, again with females on the left and males on the right. An asterisk (*) is used to designate within group differences ($p < 0.05$) in drinking compared to baseline, as evident from analysis of simple effects drawn from significant interactions in the 2 (sex) $\times$ 2 (condition) repeated measure ANOVA done separately for each test day. Difference scores, comparing at the change in preference between Lock and Free days across the experimental groups, are depicted in the right panels for consumption (**B**) and preference (**B'**), and the asterisk (*) reflects changes in drinking that differ significantly from zero. There were 9 each of male and female subjects in Experiment 3.

Fig. 4. Blood samples taken immediately following the final 2hrs of 20% EtOH access in Experiment 3, when the activity wheels were locked, yielded a range of blood EtOH concentrations, reflecting differences in consumption both within and between groups. Blood from 7 males and 9 females were analyzed.