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Chronic social isolation and chronic variable stress during early development induce later elevated ethanol intake in adult C57BL/6J mice

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Abstract

Experience with stress situations during early development can have long-lasting effects on stress and anxiety related behaviors. Importantly, this can also favor drug self-administration. These studies examined the effects of chronic social isolation and/or variable stress experiences during early development on subsequent voluntary ethanol intake in adult male and female C57BL/6J mice. The experiments were conducted to evaluate the effect of chronic isolation between weaning and adulthood (Experiment 1), chronic isolation during adulthood (Experiment 2) and chronic variable stress alone or in combination with chronic social isolation between weaning and adulthood (Experiment 3) on subsequent voluntary ethanol intake. Mice were born in our facility and were separated into two housing conditions: isolate-housed (1 mouse/cage) or group-housed (4 mice/cage), according to sex. Separate groups were isolated for 40 days starting either at time of weaning postnatal day 21 (PD 21) (early isolation, Experiments 1 and 3) or at adulthood (PD 60: late isolation, Experiment 2). The effects of housing condition on subsequent ethanol intake were assessed starting at around PD 65 in Experiments 1 and 3 or PD 105 days in Experiment 2. In Experiment 3, starting at PD 32, isolate-housed and group-housed mice were either subjected to chronic variable stress (CVS) or left undisturbed. CVS groups experienced random presentations of mild stressors for 14 days, including exposure to an unfamiliar open field, restraint, physical shaking, and forced swim, among others. All mice were tested for ethanol intake for 14 days using a 2-bottle choice (ethanol 15 % v/v vs. water) 2-hr limited access procedure. Early social isolation resulted in greater ethanol intake in comparison to the corresponding group-housed mice (Experiment 1). In contrast, social isolation during adulthood (late isolation) did not increase subsequent ethanol intake compared to the corresponding group-housed mice (Experiment 2). For mice that did not experience CVS, early social isolation resulted in greater ethanol intake compared to group-housed mice (Experiment 3). CVS subsequently resulted in a significant increase in ethanol intake in group-housed mice, but CVS failed to further increase ethanol intake in mice that experienced chronic social isolation early in life (Experiment 3). Overall, female mice consumed more ethanol than males, whether isolated (early or late) or group housed. These results indicate that early, but not late social isolation can subsequently influence ethanol consumption in C57BL/6J mice. Thus, the developmental timing of chronic social isolation appears to be an important factor in defining later effects on ethanol self-administration behavior. In addition,

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experience with CVS early in life results in elevated ethanol intake later in adulthood. Taken together, these results emphasize the important role of early stress experiences that modulate later voluntary ethanol intake during adulthood.

Keywords

Ethanol intake; adolescence; social isolation; chronic variable stress; mouse

Introduction

Many studies have reported that exposure to stressful situations early during development is a significant risk factor for future excessive alcohol (ethanol) consumption, thereby increasing the risk for dependence and alcoholism (Enoch, 2006; Schuckit and Hesselbrock, 2004; Uhart and Wand, 2009). Retrospective studies conducted with adult subjects have indicated a high correlation between adverse, stressful events early in life and ethanol drinking, especially initiation of ethanol intake during early or mid-adolescence (Dube et al., 2006). Longitudinal studies in which adolescents with a history of maltreatment were monitored for 2 years have also confirmed that childhood trauma is a potent predictor of early ethanol use (Kaufman et al., 2007).

Adolescence is a critical developmental period in which many physiological and neurobiological changes occur as an individual transitions into adulthood (Spear, 2000; Spear, 2004). Studies examining the effects of stressors during adolescence on later ethanol drinking are of importance because adolescence is a developmental period often characterized as stressful, with many individuals also experimenting with alcohol during this ontogenetic phase (Crews et al., 2007). While adolescence is often thought of as a unique period of development in humans, it has been shown that other species such as rodents and non-human primates undergo a similar ontogenetic transition from dependence to independence that includes a variety of neural, hormonal, and behavioral changes (Spear, 2000; Spear, 2004). Among rodents, adolescence is broadly defined as the period between weaning and 60 days of age (Spear, 2000).

Several animal models have been employed to better understand the relationship between stress and ethanol intake and to analyze the effects of stressful experiences early in life on future ethanol intake (Sillaber and Henniger, 2004). In particular, rodents have been widely used to evaluate developmental changes in fear- and stress-related behaviors, with chronic stress during early developmental periods inducing many behavioral changes including altered anxiety responses, locomotor activity, and aggressive behaviors (Hefner and Holmes, 2007; McCormick et al., 2007; Spear, 2000; Spear, 2004). Social isolation is a stressor that has been employed during early development to examine potential long-lasting effects on behavioral and biological responses to stress in rats (Hall et al., 1998a; Leussis and Andersen, 2008; Leussis et al., 2008) and mice (Avitsur et al., 2003; Gariépy et al., 1995; Guo et al., 2004; Zhu et al., 2006). This chronic stress situation has, indeed, been shown to have long-lasting effects on various behavioral and neural functions (Lapiz et al., 2003; Weiss et al., 2004). More directly related to the present studies, chronic social isolation early in ontogeny has been shown to alter response to several drugs of abuse in rats (Gordon, 2002; Kabbaj et al., 2002; McCormick et al., 2004), with some studies reporting increased drug self-administration later in life (Bardo et al., 2001; Ding et al., 2005; Howes et al., 2000). Other studies have been conducted to evaluate the impact of chronic stress early in development on voluntary ethanol intake later in life. Specifically, it has been shown that chronic social isolation during adolescence leads to higher ethanol self-administration in adulthood using different strains of rats (Hall et al., 1998b; Juárez and Vázquez-Cortés,

2003; Mccool and Chappell, 2009), as well as lines of rats selectively bred for high ethanol preference (Ehlers et al., 2007).

While several studies have examined the effects of stress exposure during adolescence on subsequent ethanol consumption in rats, few studies have examined the impact of chronic social isolation during adolescence using mice. To our knowledge, only two studies have attempted to address this issue. In one study, C57BL/10 and DBA1 mice were forced to consume a liquid diet containing ethanol for 14 days starting on postnatal day 30. In this study, isolate-housed mice consumed more ethanol than group-housed controls, with significantly greater mortality observed among socially isolated mice drinking the ethanol-containing liquid diet in comparison to isolated mice drinking a control liquid diet (Yanai and Ginsburg, 1976). In a more recent study that examined voluntary ethanol consumption, socially isolated male adolescent C57BL/6J mice increased their ethanol intake in adulthood when compared to socially (group) housed controls using a continuous-access two-bottle preference test [i.e., mice were allowed free-access to ethanol and water for 24 hr periods starting at postnatal day 60] (Advani et al., 2007).

The studies presented here were intended to further evaluate the effect of chronic social isolation during adolescence on later voluntary ethanol intake in C57BL/6J mice. Specifically, using a 2-bottle choice (ethanol vs. water) limited access procedure, ethanol intake in adult mice that received long-term social isolation either early in development (from weaning) (Experiment 1) or in adulthood (Experiment 2) was examined. Importantly, several studies have demonstrated that mice drinking in this limited access procedure achieve blood and brain ethanol concentrations that are physiologically relevant and correlate positively with the amount of ethanol intake recorded (Becker and Lopez, 2004; Grahame and Grose, 2003; Griffin et al., 2009).

Another goal of the current experimental series was to investigate the effect of chronic variable stress (CVS) exposure during adolescence on later voluntary ethanol intake. Similar to chronic social isolation, prior studies have shown that experience with chronic variable and unpredictable stress has long lasting effects on HPA axis regulation and future response to stress (Flak et al., 2009; Jankord and Herman, 2008; Ostrander et al., 2009). More importantly, studies conducted with rats have shown that experience with chronic variable stress affects later responsiveness to drugs of abuse such as cocaine (Lepsch et al., 2005), morphine (Molina et al., 1994) and amphetamine (Kabbaj et al., 2002; Lin et al., 2002). To our knowledge, however, the effect of chronic variable stress during adolescence on voluntary ethanol consumption later in adulthood has not yet been studied. Therefore, in Experiment 3, adolescent mice were exposed to chronic variable (and unpredictable) stress alone or in combination with social isolation and voluntary ethanol consumption during adulthood was later examined. It was predicted that both chronic stress conditions (social isolation and variable stress) administered during adolescence would induce higher levels of ethanol intake during adulthood, with the combination of these two stressors interacting to produce even greater levels of intake than either stressor alone.

Materials and Methods

Animals

Adult male and female C57BL/6 mice purchased from Jackson Laboratories (Bar Harbor, ME) were used to create a breeding colony. Two weeks after arrival to our animal facility, breeding pairs of mice were established. Mice had free access to food (Harland Teklad, Madison, WI) and water throughout all phases of the experiments except during the brief periods of stress exposure in Experiment 3. Mice were housed in a temperature and humidity-controlled animal facility under a 12-hr light/dark cycle (lights on at 0200 hr).

Husbandry was conducted once a week. Body weights of mice were recorded weekly. Once females had become noticeably pregnant, males were removed from the cage. At that point, cages were checked daily for pups. The day that pups were delivered was designated postnatal day 0 (PND-0). Litter size ranged from 4 to 7 pups. For each experiment mice were born within a 7-days period. All animals used in these experiments were weaned at PND-21. Sex was verified upon weaning, at which time mice were assigned to a particular experimental group. The number of mice assigned to each experimental group is indicated in Table 1. All experimental protocols were approved by the Institutional Animal Care and Use Committee at the Medical University of South Carolina in accordance with the guidelines of the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1996).

Experimental Design

Experiment 1: Effect of early chronic social isolation on later ethanol intake—

The experiment was based on two factors, Sex (male and female) and Housing condition (isolate- or group-housed). At weaning, mice were weighed and housed in groups (4 per cage) according to sex, or individually housed. In this, and in all subsequent experiments, precautions were taken to avoid having more than two mice from the same litter represented in a given experimental group. Moreover, mice from different litters were combined for group-housed conditions. Approximately forty days later (PND 60-65), all mice were housed individually, with daily ethanol intake then assessed 3 days later using a limited access procedure (described below). Ethanol intake was measured for 14 days. Immediately after the final intake test day in Experiment 1, blood samples (40 μ l) were collected from the retro-orbital sinus using a heparinized capillary tube. Plasma was separated by centrifugation and the ethanol concentration was measured using an Analox Instrument analyzer (Lunenburg, MA).

Experiment 2: Effect of late chronic social isolation on subsequent ethanol intake—

This experiment was also based on two factors; Sex (male and female) and Housing condition (isolate- and group-housed). At weaning, mice were group-housed (4 per cage) according to sex. At PND-60, half of the mice were separated and housed individually for 40-45 days. After this period of social isolation, all mice were individually housed and three days later, mice were given access to ethanol in their home cage. A limited access procedure (described below) was used to record ethanol intake for 14 days.

Experiment 3: Effect of early chronic social isolation and chronic variable stress on later ethanol intake—

The experiment was defined by three factors; Sex (male and female), Housing condition (isolate- and group-housed), and Stress (chronic variable stress (CVS) or no stress). At weaning half of the mice were group housed (4 per cage) according to sex while the remaining mice were singly housed. Mice were quasi-randomly assigned to each experimental group, such that no more than two mice from the same litter were placed in a given experimental group. Starting at PND-35, half of the isolate-housed and half of the group-housed mice were subjected to CVS as described below. CVS exposure was administered during 14 consecutive days. The remaining isolate-housed and group-housed mice were left undisturbed during this period of time. At PND-60-65 all mice were individually housed and three days later tested for voluntary ethanol intake using a limited access procedure for 14 days.

Procedures

Limited Access Ethanol Drinking—At 30 min before the beginning of the dark cycle (1330 hr), water bottles were removed from the home cage and replaced with two 15-ml graduated tubes that contained either a 15% (v/v) ethanol solution or water. After a 2-hr

access period, the graduated tubes were removed and replaced with the original water bottles. Position of the ethanol and water tubes was alternated on a daily basis. The amount consumed was recorded daily (± 0.1 ml), and body weights were recorded each week. Solutions were presented at room temperature and were prepared fresh each day by mixing ethanol (95%) with deionized water. Throughout all of the experiments, mice were neither food nor water deprived.

Chronic Variable Stress—Subjects that received chronic variable stress were moved to a different room at PND-32 so that the remaining (no-stress) groups of mice were not disturbed. Chronic variable stress began on PND-35 and was administered according to a randomized schedule for 14 days using different stressors or different length of exposure to the same stressor. Stressors were scheduled such that mice received a stress session in the morning and a different stressor in the afternoon with the exception of continuous light cycle for 36 hours. Mice in the group-housed condition were exposed to the same stress procedure within each cage. Stress procedures were selected based on studies previously conducted with rats (Cullinan and Wolfe, 2000; Ostrander et al., 2006) and included: *Swim*: mice were forced to swim in water (24-26° C) for 5 or 10 min; *Cold swim*: mice were forced to swim in cold water (19-20° C) for 5 or 7 min; *Movement/Vibration*: mice were placed in a standard mouse cage situated on a platform that either constantly rocked, tilted, or vibrated for 5 or 10 min; *Open field*: mice were placed in an unfamiliar open field arena for 5 or 10 min; *Open field with novel object*: mice were placed in an open field with an unfamiliar object (plastic bottle lid placed in the center of the floor) for either 5 or 10 min; *IP injection*: animals were punctured with a 26G 3/8 needle attached to an empty 1cc syringe (no fluid was delivered); *Continuous light cycle*: lights remained on for 36 continuous hrs; *White noise*: mice were exposed to white noise (75 dB) in a separate room for 30 min; *Slanted wire floor*: mice were placed in a cage with an angled grid floor (25°) for 15 or 30 min; and *Restraint*: animals were placed in plastic restraint holders for 25 min.

At PND-50, all chronically stressed mice were returned to the colony room where the non-stressed mice were housed. At PND-60 all animals were individually housed and three days later daily ethanol intake was examined as described above.

Data analyses

Ethanol intake (mls and g/kg), body weight (grams) and water intake (mls) served as dependent variables in these studies. Data were analyzed using between-within factorial Analyses of Variance (ANOVA). Details about each factorial analysis are presented in the results section for each experiment. Whenever a factor or interaction of factors yielded a significant effect in the overall ANOVA, further analysis was conducted in separate two- or one-way ANOVA followed by pair-wise post-hoc comparisons with Bonferroni corrections.

Results

Experiment 1: Effect of early chronic social isolation on subsequent ethanol intake

Body weights recorded at the beginning of the ethanol intake testing period indicated that although males were heavier than females [$F(1,42)= 124.55$, $p< 0.001$], chronic social isolation during adolescence did not significantly affect body weight at adulthood (Table 1).

For analysis of ethanol intake, ANOVA was conducted with Sex (male, female) and Housing (isolate-, group-housed) as between factors and Days as a repeated measure. Results indicated that overall, ethanol intake was significantly greater in females compared to males [$F(1,42)= 30.04$, $p<0.001$], isolate-housed mice consumed more ethanol than group-housed mice [$F(1,42)= 23.86$], and ethanol intake varied across days [$F(13,546)=$

16.56]; all $p < 0.001$]. The three-way interaction between these factors was also significant [$F(13,546) = 2.70$, $p < 0.01$].

Separate two-way ANOVAs were conducted for each sex in order to analyze the effect of housing condition on ethanol intake. For males, a significant main effect of Housing indicated that overall, isolate-housed mice consumed significantly more ethanol than group-housed mice [$F(1,22) = 18.95$, $p < 0.001$]. A significant Housing $\times$ Days interaction [$F(13,286) = 3.11$, $p < 0.001$] followed by post-hoc analyses revealed that adult mice socially isolated during adolescence consumed more ethanol than group-housed mice on all days of the test period except days 4, 12 and 13 (Figure 1A). Analysis of females also indicated a significant main effect of Housing [$F(1,20) = 6.22$, $p < 0.025$] and a significant Housing $\times$ Days interaction [$F(13,260) = 3.99$, $p < 0.025$]. Post-hoc analyses indicated that, while isolate-housed female mice consumed more ethanol than group-housed mice, this difference was significant only during test days 1, 8 and 10 (Figure 1B). The analysis of ethanol intake in ml showed almost identical results that the analysis of ethanol intake in g/kg. Analysis of ethanol intake in ml indicated a significant main effect of Housing [$F(1,42) = 24.78$, $p < 0.001$], Days [$F(13,546) = 17.46$, $p < 0.001$], and significant interactions between Housing and Days [$F(13,546) = 4.72$, $p < 0.001$] and Housing, Days and Sex [$F(13,546) = 2.68$, $p < 0.01$]. However, this analysis failed to indicate a significant main effect of Sex [$F(1,42) = 2.09$, $p = 0.16$] indicating that sex differences in g/kg of ethanol intake were due to a lower body weight in females (data not shown). Water intake was not affected by sex [$F(1,42) = 0.51$, $p = 0.48$] or housing conditions [$F(1,42) = 0.71$, $p = 0.40$]. The ANOVA for water intake indicated only a significant main effect of days related to some daily variations on water intake [$F(13,546) = 5.60$, $p < 0.001$] (data not shown).

Blood ethanol concentrations (BEC) recorded at the end of the experiment (following the last limited access drinking session) were in a range suggesting that intake was physiologically relevant for both males (136.10 ± 17.53 mg/dl) and females (116.50 ± 15.88 mg/dl) (values are mean $\pm$ s.e.m. collapsed across housing conditions). Analysis of BEC values indicated no significant differences related to housing condition. Additionally, analysis indicated that BEC registered immediately after the 2-hr drinking session was significantly correlated with the amount of ethanol (g/kg) consumed ($r^2 = 0.35$ for males and $r^2 = 0.61$ for females, both $p < 0.05$).

Experiment 2: Effect of late chronic social isolation on subsequent ethanol intake

Body weight recorded at the beginning of the intake test revealed that, while males were heavier than females [$F(1,36) = 148.43$, $p < 0.001$], chronic social isolation during adulthood did not alter subsequent weight gain compared to group-housed controls (Table 1).

Analysis of ethanol intake indicated a significant main effect of Sex, with females drinking more ethanol than males [$F(1,36) = 7.57$, $p < 0.01$]. While there was only a strong trend for housing condition to influence drinking [$F(1,36) = 3.77$, $p = 0.06$], the two-way interaction between Housing and Days was significant [$F(13,468) = 1.80$, $p < 0.05$].

Separate two-way ANOVAs were conducted for each sex. For males, ANOVA indicated only a significant main effect of Days [$F(13,234) = 4.23$, $p < 0.001$], with no significant main effect or interactions involving Housing (Figure 2A). Analysis of female data indicated a significant main effect of Days [$F(13,234) = 7.92$, $p < 0.025$], as well as the Housing $\times$ Days interaction [$F(13,234) = 2.18$, $p < 0.025$]. Post-hoc comparisons based on the two-way interaction indicated that group-housed female mice consumed significantly more ethanol than isolate-housed mice on days 6, 8, and 10 of the testing period (Figure 2B). Analyses of ethanol intake in ml showed a similar profile of results to those of ethanol intake. Only a significant main effect of days was observed [$F(13,468) = 12.62$, $p < 0.001$] related to some

daily overall variations on ethanol intake. However, the analysis failed to show significant differences in intake between males and females [$F(1,36)=0.03$, $p=0.86$] (data not shown). Analysis of water intake showed no effects of chronic isolation in males and females [$F(1,36)=0.54$, $p=0.47$] (data not shown).

Experiment 3: Effect of early chronic social isolation alone and in combination with chronic variable stress on subsequent ethanol intake

Analysis of body weight registered at the beginning of ethanol intake testing indicated that, overall, males were heavier than females [$F(1,69)=15.71$, $p<0.001$]. Exposure to chronic social isolation, CVS, or the combination of these stressors during adolescence, however, did not significantly affect adult body weight.

The effects of social isolation and CVS alone or administered in combination during adolescence on later adult ethanol drinking was analyzed by ANOVA, with Sex, Housing (isolate- or group-housed) and Stress (CVS or control) as between-subject factors and Days as a repeated measure. Results revealed significant main effects of Sex [$F(1,67)=43.44$, $p<0.001$], Housing [$F(1,67)=4.63$, $p<0.05$], and Days [$F(13,871)=23.01$, $p<0.01$]. The ANOVA also revealed significant Housing $\times$ Stress [$F(1,67)=4.38$, $p<0.05$], Housing $\times$ Days [$F(13,871)=3.76$, $p<0.01$], and Stress $\times$ Days [$F(13,871)=2.41$, $p<0.01$] interactions, as well as the three-way interaction term (Sex $\times$ Housing $\times$ Days) [$F(13,871)=1.79$, $p<0.05$]. Therefore, to further analyze the effect of housing and stress, and to be consistent with the analyses of the previous experiments, additional analyses with Housing and Stress as between-subject factors and Days as a repeated measure were conducted separately for male and female mice.

In males, a significant main effect of Days [$F(13,377)=9.32$, $p<0.05$], and significant Housing $\times$ Days [$F(13,377)=2.34$, $p<0.01$] and Stress $\times$ Days [$F(13,377)=1.83$, $p<0.05$] interactions were observed. Post-hoc analyses indicated that, similar to Experiment 1, chronic social isolation (without CVS) induced higher voluntary ethanol intake during the first 3 days of testing compared to group-housed (non-stressed) males. Additionally, CVS alone (in male mice that were group-housed) during adolescence resulted in significantly greater ethanol intake on days 1, 3, 11, 12, and 13 compared to non-stressed group-housed controls. Furthermore, male mice that received a combination of social isolation and CVS treatment during adolescence consumed significantly more ethanol than group-housed controls during days 1, 4, 7, and 11 of testing. Ethanol intake among these same male mice, however, was not significantly different from mice that experienced chronic social isolation alone (Figure 3). For females, ANOVA indicated a significant main effect of Housing [$F(1,38)=4.76$, $p<0.05$] and Days [$F(13,494)=15.86$, $p<0.001$] as well as a significant interaction between these factors [$F(13,494)=3.43$, $p<0.001$], but failed to show a significant effect of CVS. Post-hoc analyses indicated that, similar to Experiment 1, females that experienced chronic social isolation during adolescence consumed significantly more than group-housed controls during days, 1, 3, 5, 6, 7, 8, and 9, regardless of stressor condition (Figure 3).

Finally, evaluation of ethanol intake in ml showed a similar profile of results but did not show significant differences in intake between males and females [$F(1,67)=0.70$, $p=0.41$] ((data not shown). Also, voluntary water intake (data not shown) was not significantly influenced by sex [$F(1,67)=1.90$, $p=0.17$], housing [$F(1,67)=0.74$, $p=0.39$], or stressor variables [$F(1,67)=1.71$, $p=0.20$].

Discussion

Results from these studies indicate that social isolation during the adolescent period significantly affected voluntary ethanol intake later in adulthood. Male and female C57BL/6J mice housed in isolation for 40 days after weaning (encompassing the broad period of adolescence in mice) consumed significantly more ethanol than mice group-housed during the same developmental period (Experiment 1). However, when the social isolation treatment was administered for the same duration during adulthood, subsequent ethanol intake was not altered relative to consumption in the corresponding group-housed controls (Experiment 2). Furthermore, Experiment 3 showed that chronic variable stress exposure during adolescence also was effective in later inducing increased ethanol intake, with the combination of social isolation and chronic variable stress not resulting in intake levels significantly greater than when mice were exposed to either stressor alone.

The present results are in agreement with other studies showing that social isolation early in ontogeny (i.e., starting at the time of weaning) can impact later ethanol intake. Specifically, several studies have demonstrated that long-term isolate-housing at weaning increases later consumption of ethanol in rats (Hall et al., 1998b; Schenk et al., 1990) or mice (Advani et al., 2007). These results also are in agreement with other studies employing various stressors during early development. For instance, maternal separation or early weaning procedures have been reported to produce similar increases in ethanol consumption in rats (Fahlke et al., 1997; Huot et al., 2001; Lancaster, 1998; Rockman et al., 1988; Roman et al., 2005), and mice (Advani et al., 2007; Cruz et al., 2008). In another study, 10 daily exposures to foot-shock stress during adolescence in mice genetically selected for their high preference for ethanol (HAP mice) resulted in augmented anxiety-related behavior as well as elevated ethanol intake during adulthood (Chester et al., 2008). Collectively, these studies corroborate the present findings that indicate social isolation and CVS during adolescence produces increased ethanol consumption later in adulthood.

Results from Experiment 1 demonstrated that chronic social isolation (40 days) beginning at weaning resulted in elevated ethanol intake for males and females. In Experiment 2, however, the same duration of social isolation starting at young adulthood (PND-60-65) did not significantly influence voluntary ethanol intake later in life in males, with a mild, yet significant reduction in intake for females. To our knowledge, there are no previous reports of reduced ethanol intake in adult females after chronic social isolation. It is interesting that the effect of chronic social isolation during adolescence has an opposite effect than during adulthood. It remains to be evaluated whether after full maturity the effects of social isolation can be different for male and female mice. This is an issue that deserves further consideration. In both cases, assessment of ethanol drinking was determined following an equivalent interval of time after the isolation manipulation. Thus, the effect of chronic social isolation on ethanol consumption appears to depend on the developmental period in which the stress is experienced. This result is in agreement with another study in rats showing that chronic social isolation during the adolescent period, but not during adulthood, produced increased ethanol intake (Schenk et al., 1990). Several other reports have demonstrated that long-term isolation beginning at weaning or adolescence results in significant increases in ethanol intake in rats (Deehan et al., 2007; Hall et al., 1998b; McCool and Chappell, 2009; Wolffgramm and Heyne, 1991) and mice (Juárez and Vázquez-Cortés, 2003), although most studies have not included a group in which isolation was initiated during adulthood and these results are not consistent (Lodge and Lawrence, 2003; Rockman et al., 1988).

Previous studies examining the impact of isolate-housing during adulthood on ethanol consumption have produced mixed results. For example, some studies have shown that long-term social isolation decreases intake (Sprague and Maickel, 1994). In contrast, other studies

using rats have shown that a period of long-term social isolation results in an increase in ethanol consumption (Nash and Maickel, 1988; Winkler et al., 1995; Wolffgramm and Heyne, 1991). Studies conducted with Wistar rats isolated during adulthood also have reported disparate results depending on the duration of the isolation period and age of the rats (Wolffgramm, 1990; Yoshimoto et al., 2003). Furthermore, an important influence of genetics has been demonstrated in a study where chronic social isolation during adulthood for 90 days resulted in elevated ethanol intake in rats selectively bred for high ethanol preference (P rats) but not in lines selected for low ethanol preference (NP rats) (Ehlers et al., 2007). Taken together, these results indicate that numerous variables including procedural differences, as well as genetic (strain) differences, may account for disparate results in effects of social isolation during adulthood on ethanol intake across studies. Also, the discrepancy in results from Experiment 2 in the present study and results obtained in prior studies with adult rats may relate to differences in the impact of social isolation across species. Rats may be more sensitive to chronic social isolation during adulthood than mice and, hence, the impact of this stress manipulation on ethanol intake may be different (Krohn et al., 2006; Miczek et al., 2008).

Results obtained in Experiment 3 replicated findings from Experiment 1, indicating that chronic social isolation during adolescence (between weaning and early adulthood) significantly increases voluntary ethanol intake. Additionally, group-housed mice that experienced fourteen days of CVS during adolescence also showed elevated voluntary ethanol intake in adulthood. CVS has been extensively studied in adult rat models and has been demonstrated to produce long-lasting effects on stress axis function, as well as the behavioral response to stress and drug challenges (Isgor et al., 2004; Jankord and Herman, 2008; Molina et al., 1994; Ostrander et al., 2006; Zurita et al., 2000). Of relevance to the present study, CVS treatment during adolescence has been shown to modify subsequent response to various drugs of abuse during adulthood. For example, studies conducted with adolescent rats have indicated that CVS exposure can inhibit sensitization to the behavioral effects of repeated amphetamine administration (Kabbaj et al., 2002) and also increase the magnitude of cocaine-induced locomotor activity (Lepsch et al., 2005). However, to our knowledge, the effects of CVS treatment administered either during the adolescent period or during adulthood on ethanol intake has not been reported. While results from Experiment 3 represent novel data relating exposure to CVS during adolescence on voluntary ethanol intake later in life, the question still remains as to whether the effects of CVS on ethanol drinking are specific to the adolescent period.

In Experiment 3, there was no evidence of an additive or synergistic effect of chronic social isolation and CVS on voluntary ethanol intake. It is possible that the method used to evaluate ethanol intake did not allow for this to be observed due to a possible ceiling effect. The level of intake reached by subjects that experienced either CVS or chronic isolation is similar to the level of intake reported in prior studies that evaluated voluntary ethanol intake in ethanol-dependent mice using this same limited access procedure (Becker and Lopez, 2004; Griffin et al., 2009). Nevertheless, use of a different ethanol concentration or a different method of assessment (e.g., operant self-administration) might unveil a potential interactive effect of combining these stressors during early development.

Sex differences in ethanol consumption were noted in each of the present experiments, with female mice drinking significantly more ethanol than males. This effect was most apparent when ethanol intake was expressed as a function of body weight, primarily due to the significantly lower body weight of female mice (male and female mice did not differ in the volume of ethanol intake in these experiments). Similar sex-related differences in ethanol consumption have been demonstrated in studies using mice (i.e., Lopez and Becker, 2003; Middaugh and Kelley, 1999; Middaugh et al., 1999). Finally, while there were some sex

differences across treatment conditions in the present study, overall chronic social isolation and/or CVS during early development produced a similar increase in ethanol intake during adulthood in males and females.

In summary, these results indicate that mice that experience chronic social isolation early in development, as opposed to during adulthood, show a significant increase in voluntary ethanol intake during adulthood. In addition, repeated exposure to unpredictable variable stress during adolescence also results in heightened voluntary ethanol intake. Based on published evidence related to early stress experience and ethanol intake it can be argued that chronic stress experiences have long lasting effects on behavioral and biological response to stress which in turn may favor higher ethanol intake. Future studies will evaluate mechanisms by which chronic stress exposure in young vs. adult mice contribute/drive increased voluntary ethanol consumption.

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References

- Advani T, Hensler J, Koek W. Effect of early rearing conditions on alcohol drinking and 5-HT1A receptor function in C57BL/6J mice. *Int J Neuropsychopharmacol*. 2007; 10:595–607. [PubMed: 17134528]
- Avitsur R, Stark JL, Dhabhar FS, Kramer KA, Sheridan JF. Social experience alters the response to social stress in mice. *Brain Behav Immun*. 2003; 17:426–437. [PubMed: 14583234]
- Bardo MT, Klebaur JE, Valone JM, Deaton C. Environmental enrichment decreases intravenous self-administration of amphetamine in female and male rats. *Psychopharmacology*. 2001; 155:278–284. [PubMed: 11432690]
- Becker HC, Lopez MF. Increased ethanol drinking after repeated chronic ethanol exposure and withdrawal experience in C57BL/6 mice. *Alcohol Clin Exp Res*. 2004; 28:1829–1838. [PubMed: 15608599]
- Chester J, Barrenha G, Hughes M, Keuneke K. Age- and sex-dependent effects of footshock stress on subsequent alcohol drinking and acoustic startle behavior in mice selectively bred for high-alcohol preference. *Alcohol Clin Exp Res*. 2008; 32:1782–1794. [PubMed: 18652593]
- 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]
- Cruz F, Quadros I, Planeta CdaS, Miczek K. Maternal separation stress in male mice: long-term increases in alcohol intake. *Psychopharmacology*. 2008; 201:459–468. [PubMed: 18766329]
- Cullinan WE, Wolfe TJ. Chronic stress regulates levels of mRNA transcripts encoding beta subunits of the GABA(A) receptor in the rat stress axis. *Brain Res*. 2000; 887:118–124. [PubMed: 11134596]
- Deehan GA Jr, Cain ME, Kiefer SW. Differential rearing conditions alter operant responding for ethanol in outbred rats. *Alcohol Clin Exp Res*. 2007; 31:1692–1698. [PubMed: 17651466]
- Ding Y, Kang L, Li B, Ma L. Enhanced cocaine self-administration in adult rats with adolescent isolation experience. *Pharmacol Biochem Behav*. 2005; 82:673–677. [PubMed: 16387352]
- Dube SR, Miller JW, Brown DW, Giles WH, Felitti VJ, Dong M, Anda RF. Adverse childhood experiences and the association with ever using alcohol and initiating alcohol use during adolescence. *The Journal of adolescent health : official publication of the Society for Adolescent Medicine*. 2006; 38:444.e441–410. [PubMed: 16549308]
- Ehlers CL, Walker BM, Pian JP, Roth JL, Slawecki CJ. Increased alcohol drinking in isolate-housed alcohol-preferring rats. *Behav Neurosci*. 2007; 121:111–119. [PubMed: 17324055]
- Enoch MA. Genetic and environmental influences on the development of alcoholism: resilience vs risk. *Ann N Y Acad Sci*. 2006; 1094:193–201. [PubMed: 17347351]
- Fahlke C, Hård E, Eriksson CJ. Effects of early weaning and social isolation on subsequent alcohol intake in rats. *Alcohol*. 1997; 14:175–180. [PubMed: 9085719]

- Flak JN, Ostrander MM, Tasker JG, Herman JP. Chronic stress-induced neurotransmitter plasticity in the PVN. *J Comp Neurol*. 2009; 517:156–165. [PubMed: 19731312]
- Gariépy JL, Gendreau PL, Mailman RB, Tancer M, Lewis MH. Rearing conditions alter social reactivity and D1 dopamine receptors in high- and low-aggressive mice. *Pharmacol Biochem Behav*. 1995; 51:767–773. [PubMed: 7675857]
- Gordon HW. Early environmental stress and biological vulnerability to drug abuse. *Psychoneuroendocrinology*. 2002; 27:115–126. [PubMed: 11750773]
- Grahame NJ, Grose AM. Blood alcohol concentrations after scheduled access in high-alcohol-preferring mice. *Alcohol*. 2003; 31:99–104. [PubMed: 14615017]
- Griffin WC, Lopez MF, Yanke AB, Middaugh LD, Becker HC. Repeated cycles of chronic intermittent ethanol exposure in mice increases voluntary ethanol drinking and ethanol concentrations in the nucleus accumbens. *Psychopharmacology*. 2009; 201:569–580. [PubMed: 18791704]
- Guo M, Wu CF, Liu W, Yang JY, Chen D. Sex difference in psychological behavior changes induced by long-term social isolation in mice. *Prog Neuropsychopharmacol Biol Psychiatry*. 2004; 28:115–121. [PubMed: 14687865]
- Hall FS, Huang S, Fong GW, Pert A, Linnoila M. Effects of isolation-rearing on locomotion, anxiety and responses to ethanol in Fawn Hooded and Wistar rats. *Psychopharmacology*. 1998a; 139:203–209. [PubMed: 9784074]
- Hall FS, Huang S, Fong GW, Pert A, Linnoila M. Effects of isolation-rearing on voluntary consumption of ethanol, sucrose and saccharin solutions in Fawn Hooded and Wistar rats. *Psychopharmacology*. 1998b; 139:210–216. [PubMed: 9784075]
- Hefner K, Holmes A. Ontogeny of fear-, anxiety- and depression-related behavior across adolescence in C57BL/6J mice. *Behav Brain Res*. 2007; 176:210–215. [PubMed: 17098297]
- Howes SR, Dalley JW, Morrison CH, Robbins TW, Everitt BJ. Leftward shift in the acquisition of cocaine self-administration in isolation-reared rats: relationship to extracellular levels of dopamine, serotonin and glutamate in the nucleus accumbens and amygdala-striatal FOS expression. *Psychopharmacology*. 2000; 151:55–63. [PubMed: 10958117]
- Huot RL, Thirivikraman KV, Meaney MJ, Plotsky PM. Development of adult ethanol preference and anxiety as a consequence of neonatal maternal separation in Long Evans rats and reversal with antidepressant treatment. *Psychopharmacology*. 2001; 158:366–373. [PubMed: 11797057]
- Isgor C, Kabbaj M, Akil H, Watson SJ. Delayed effects of chronic variable stress during peripubertal-juvenile period on hippocampal morphology and on cognitive and stress axis functions in rats. *Hippocampus*. 2004; 14:636–648. [PubMed: 15301440]
- Jankord R, Herman JP. Limbic regulation of hypothalamo-pituitary-adrenocortical function during acute and chronic stress. *Ann N Y Acad Sci*. 2008; 1148:64–73. [PubMed: 19120092]
- Juárez J, Vázquez-Cortés C. Alcohol intake in social housing and in isolation before puberty and its effects on voluntary alcohol consumption in adulthood. *Dev Psychobiol*. 2003; 43:200–207. [PubMed: 14558042]
- Kabbaj M, Isgor C, Watson SJ, Akil H. Stress during adolescence alters behavioral sensitization to amphetamine. *Neuroscience*. 2002; 113:395–400. [PubMed: 12127096]
- Kaufman J, Yang BZ, Douglas-Palumberi H, Crouse-Artus M, Lipschitz D, Krystal JH, Gelernter J. Genetic and environmental predictors of early alcohol use. *Biol Psychiatry*. 2007; 61:1228–1234. [PubMed: 17123474]
- Krohn T, Sorensen D, Ottesen J, Hansen A. The effects of individual housing on mice and rats: a review. *Animal Welfare*. 2006; 15:343–352.
- Lancaster FE. Sex differences in voluntary drinking by Long Evans rats following early stress. *Alcohol Clin Exp Res*. 1998; 22:830–836. [PubMed: 9660308]
- Lapiz MD, Fulford A, Muchimapura S, Mason R, Parker T, Marsden CA. Influence of postweaning social isolation in the rat on brain development, conditioned behavior, and neurotransmission. *Neurosci Behav Physiol*. 2003; 33:13–29. [PubMed: 12617300]
- Lepsch LB, Gonzalo LA, Magro FJ, Delucia R, Scavone C, Planeta CS. Exposure to chronic stress increases the locomotor response to cocaine and the basal levels of corticosterone in adolescent rats. *Addict Biol*. 2005; 10:251–256. [PubMed: 16109586]

- Leussis M, Andersen S. Is adolescence a sensitive period for depression? Behavioral and neuroanatomical findings from a social stress model. *Synapse*. 2008; 62:22–30. [PubMed: 17957735]
- Leussis M, Lawson K, Stone K, Andersen S. The enduring effects of an adolescent social stressor on synaptic density, part II: Poststress reversal of synaptic loss in the cortex by adinazolam and MK-801. *Synapse*. 2008; 62:185–192. [PubMed: 18081181]
- Lin D, Bruijnzeel AW, Schmidt P, Markou A. Exposure to chronic mild stress alters thresholds for lateral hypothalamic stimulation reward and subsequent responsiveness to amphetamine. *Neuroscience*. 2002; 114:925–933. [PubMed: 12379248]
- Lodge DJ, Lawrence AJ. The CRF1 receptor antagonist antalarmin reduces volitional ethanol consumption in isolation-reared fawn-hooded rats. *Neuroscience*. 2003; 117:243–247. [PubMed: 12614667]
- Lopez MF, Becker HC. Gender differences in ethanol intake prior to and following repeated chronic ethanol exposure and withdrawal in C57BL/6J mice. *Alcohol Clin Exp Res*. 2003; 27:15A.
- Mccool B, Chappell A. Early social isolation in male Long-Evans rats alters both appetitive and consummatory behaviors expressed during operant ethanol self-administration. *Alcohol Clin Exp Res*. 2009; 33:273–282. [PubMed: 19032581]
- McCormick CM, Merrick A, Secen J, Helmreich DL. Social instability in adolescence alters the central and peripheral hypothalamic-pituitary-adrenal responses to a repeated homotypic stressor in male and female rats. *J Neuroendocrinol*. 2007; 19:116–126. [PubMed: 17214874]
- McCormick CM, Robarts D, Gleason E, Kelsey JE. Stress during adolescence enhances locomotor sensitization to nicotine in adulthood in female, but not male, rats. *Horm Beh*. 2004; 46:458–466.
- Miczek K, Yap JJ, Covington HE. Social stress, therapeutics and drug abuse: preclinical models of escalated and depressed intake. *Pharmacol Ther*. 2008; 120:102–128. [PubMed: 18789966]
- Middaugh LD, Kelley BM. Operant ethanol reward in C57BL/6 mice: influence of gender and procedural variables. *Alcohol*. 1999; 17:185–194. [PubMed: 10231166]
- Middaugh LD, Kelley BM, Bandy AL, McGroarty KK. Ethanol consumption by C57BL/6 mice: influence of gender and procedural variables. *Alcohol*. 1999; 17:175–183. [PubMed: 10231165]
- Molina VA, Heyser CJ, Spear LP. Chronic variable stress enhances the stimulatory action of a low dose of morphine: reversal by desipramine. *Eur J Pharmacol*. 1994; 260:57–64. [PubMed: 7957627]
- Nash JF Jr, Maickel RP. The role of the hypothalamic-pituitary-adrenocortical axis in post-stress induced ethanol consumption by rats. *Prog Neuropsychopharmacol Biol Psych*. 1988; 12:653–671.
- Ostrander MM, Ulrich-Lai YM, Choi DC, Flak JN, Richtand NM, Herman JP. Chronic stress produces enduring decreases in novel stress-evoked c-fos mRNA expression in discrete brain regions of the rat. *Stress*. 2009; 12:469–477. [PubMed: 20102318]
- Ostrander MM, Ulrich-Lai YM, Choi DC, Richtand NM, Herman JP. Hypoactivity of the hypothalamo-pituitary-adrenocortical axis during recovery from chronic variable stress. *Endocrinology*. 2006; 147:2008–2017. [PubMed: 16396985]
- Rockman GE, Hall AM, Markert LE, Glavin GB. Influence of rearing conditions on voluntary ethanol intake and response to stress in rats. *Behav Neural Biol*. 1988; 49:184–191. [PubMed: 3365187]
- Roman E, Gustafsson L, Hyytiä P, Nylander I. Short and prolonged periods of maternal separation and voluntary ethanol intake in male and female ethanol-preferring AA and ethanol-avoiding ANA rats. *Alcohol Clin Exp Res*. 2005; 29:591–601. [PubMed: 15834224]
- Schenk S, Gorman K, Amit Z. Age-dependent effects of isolation housing on the self-administration of ethanol in laboratory rats. *Alcohol*. 1990; 7:321–326. [PubMed: 2390208]
- Schuckit MA, Hesselbrock V. Alcohol Dependence and Anxiety Disorders: What Is the Relationship? *Focus*. 2004; 2:440–453.
- Sillaber I, Henniger MS. Stress and alcohol drinking. *Ann Med*. 2004; 36:596–605. [PubMed: 15768831]
- Spear LP. The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev*. 2000; 24:417–463. [PubMed: 10817843]
- Spear LP. Adolescent brain development and animal models. *Ann N Y Acad Sci*. 2004; 1021:23–26. [PubMed: 15251870]

- Sprague JE, Maickel RP. Effects of stress and ebitatide (Hoe-427) on free-choice ethanol consumption: comparison of Lewis and Sprague-Dawley rats. *Life Sci.* 1994; 55:873–878. [PubMed: 8072388]
- Uhart M, Wand G. Stress, alcohol and drug interaction: an update of human research. *Addict Biol.* 2009; 14:43–64. [PubMed: 18855803]
- Weiss IC, Pryce CR, Jongen-Relo AL, Nanz-Bahr NI, Feldon J. Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat. *Behav Brain Res.* 2004; 152:279–295. [PubMed: 15196796]
- Winkler A, Roske I, Furkert J, Fickel J, Melzig MF. Effects of voluntary ethanol ingestion on the POMC gene expression in the rat pituitary and on the plasma beta-endorphin content. *Alcohol Alcohol.* 1995; 30:231–238. [PubMed: 7662043]
- Wolffgramm J. Free choice ethanol intake of laboratory rats under different social conditions. *Psychopharmacology (Berl).* 1990; 101:233–239. [PubMed: 2349365]
- Wolffgramm J, Heyne A. Social behavior, dominance, and social deprivation of rats determine drug choice. *Pharmacol Biochem Behav.* 1991; 38:389–399. [PubMed: 2057508]
- Yanai J, Ginsburg BE. Increased sensitivity to chronic ethanol in isolated mice. *Psychopharmacologia.* 1976; 46:185–189. [PubMed: 986055]
- Yoshimoto K, Komura S, Yasuhara M. The age of drinking onset and housing condition influences rat alcohol drinking behavior. *Nihon Arukoru Yakubutsu Igakkai Zasshi.* 2003; 38:335–340. [PubMed: 14515742]
- Zhu SW, Pham TM, Aberg E, Brené S, Winblad B, Mohammed AH, Baumans V. Neurotrophin levels and behaviour in BALB/c mice: impact of intermittent exposure to individual housing and wheel running. *Behav Brain Res.* 2006; 167:1–8. [PubMed: 16343654]
- Zurita A, Martijena I, Cuadra G, Brandão ML, Molina V. Early exposure to chronic variable stress facilitates the occurrence of anhedonia and enhanced emotional reactions to novel stressors: reversal by naltrexone pretreatment. *Behav Brain Res.* 2000; 117:163–171. [PubMed: 11099770]

Figure 1.

Daily voluntary ethanol intake (g/kg) in (A) male and (B) female mice that were isolate-housed or group-housed for 40 days after weaning. Values are mean $\pm$ SEM. * indicates a significant difference between housing conditions for a particular measurement day ($p < 0.05$).

Figure 2. Daily voluntary ethanol intake (g/kg) in (A) male and (B) female mice that were group-housed or isolate-housed (for 40 days starting at postnatal day 60). Values are mean $\pm$ SEM. * indicates a significant difference between housing conditions ($p < 0.05$).

Figure 3.

Daily voluntary ethanol intake (g/kg) in male and female mice that were isolate- or group-housed at weaning. Half of the animals from each housing condition were also exposed to chronic variable stress (CVS) during adolescence (postnatal days 32 to 45), while the remaining animals received no stress (NS). Values are mean $\pm$ SEM.

* indicates significant difference between group-housed and isolated controls ($p < 0.05$)

indicates significant difference between CVS mice and controls mice ($p < 0.05$)

† indicates significant difference between isolate-housed CVS mice and group-housed controls ($p < 0.05$)

Table 1

Body weights of male and female animals from Experiments 1, 2 and 3. Values are mean $\pm$ SEM (n).

		Experiment 1 (Isolation started at weaning)	Experiment 2 (Isolation started at PND 60)	Experiment 3 (Isolation at weaning and CVS)
No CVS	Male	IH 22.82 $\pm$ 0.40 (10)	27.50 $\pm$ 0.9 (10)	22.21 $\pm$ 0.08 (7)
		GH 23.85 $\pm$ 0.39 (14)	26.89 $\pm$ 0.44 (10)	24.24 $\pm$ 0.49 (8)
	Female	IH 19.23 $\pm$ 0.23 (13)	21.68 $\pm$ 0.29 (10)	18.17 $\pm$ 0.44 (9)
		GH 19.44 $\pm$ 0.35 (9)	21.09 $\pm$ 0.20 (10)	19.45 $\pm$ 0.39 (10)
CVS	Male	IH		22.22 $\pm$ 0.26 (10)
		GH		23.74 $\pm$ 0.63 (11)
	Female	IH		18.33 $\pm$ 0.14 (8)
		GH		18.12 $\pm$ 0.19 (12)

IH: Indicates that subjects were isolate-housed
 GH: Indicates that subjects were group-housed (4 per cage)
 CVS: Animals were exposed to chronic variable stress