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Impact of social isolation and enriched environment during adolescence on voluntary ethanol intake in C57BL/6J mice

Marcelo F. Lopez, Ph.D.^{1,*} and Kathy Laber, DVM MS DACLAM²

¹Medical University of South Carolina, 171 Ashley Avenue, Charleston, S.C. 29425

²VA Medical Center, 114 Doughty Street, Charleston, S.C. 29425, and Comparative Medicine Branch NIEHS; PO Box 12333 MD-C1-05, Research Triangle Park NC 27709

Abstract

This study was designed to determine the impact of an enriched environment in a previously established stress model of isolation during early development that induces high alcohol (ethanol) self-administration. The study was conducted with male and female C57BL/6J mice housed in isolation or in groups that were either provided or withheld enrichment during adolescence. The impact of these housing conditions was assessed during adulthood by measuring weight gain, quantifying voluntary ethanol intake, measuring plasma corticosterone levels, and assessing anxiety-like behavior. Results showed that, regardless of sex, mice that were single-housed during adolescence showed a significant increase in voluntary ethanol intake, which was not observed in isolated mice that were provided with nesting material during adolescence (compared to group-housed non-enriched control group). Basal corticosterone was not affected by housing, enrichment conditions, or sex. Corticosterone levels did not relate to levels of voluntary ethanol intake. However, corticosterone levels were higher after three weeks of ethanol intake. Surprisingly, mice that were group-housed during adolescence showed higher levels of anxiety-like behavior in the light/dark test. Overall, these results indicate that housing conditions during a critical developmental period can significantly modulate voluntary ethanol intake later in life.

Keywords

Alcohol; Isolation; Adolescence; Enriched Environment

Introduction

The experience of stressful situations during early development is a significant risk factor for future excessive alcohol (ethanol) consumption, increasing the risk for dependence and alcoholism (Becker et al. 2011; Enoch 2006, Schuckit and Hesselbrock, 2004; Uhart and Wand, 2009). Retrospective (Dube et al., 2006) as well as longitudinal (Kaufman et al.

*Corresponding Author. Marcelo F. Lopez, Ph.D., Medical University of South Carolina, Center for Drug & Alcohol Programs, 67 President Street, MSC 861, Charleston, SC 29425, Phone: 843-789-6772, Fax: 843-792-7353, lopezm@musc.edu.

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2007) studies have shown a strong relationship between the experience of stressful events early in life and ethanol drinking, especially initiation of ethanol intake during early or mid-adolescence.

Adolescence is a critical developmental period; many physiological and neurobiological changes happen as individuals transition into adulthood (Spear 2000, 2004). Several studies have examined the effects of stressors during adolescence on later ethanol drinking 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 considered 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 neural, hormonal, and behavioral changes (Spear 2000, 2004).

Several animal models have been employed to understand the relationship between stressful experiences early in life on future ethanol intake (Becker et al. 2011; Sillaber and Henniger, 2004). Social isolation during early development has long-lasting effects on behavioral and biological responses to stress in rats (Hall et al. 1998a, Leussis et al. 2008, Leussis and Andersen, 2008) and mice (Avitsur et al. 2003, Gariépy et al. 1995, Guo et al. 2004, Zhu et al. 2006). Chronic isolation during early development also alters the 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). Importantly, chronic social isolation during adolescence has been linked to higher ethanol self-administration in adulthood using different strains of rats (e.g. Hall et al. 1998b, Juarez and Vazquez Cortes 2003, McCool and Chappell, 2009), lines of rats selectively bred for high ethanol preference (e.g. Ehlers et al. 2007), and mice (Advani et al. 2007; Yanai and Ginsburg, 1976). A recent study has shown that chronic social isolation during adolescence results in higher voluntary ethanol intake in C57BL/6J mice during adulthood (Lopez et al. 2011). In contrast, chronic social isolation during adulthood did not increase voluntary ethanol intake; this indicates that the effect of chronic social isolation on voluntary ethanol intake is specific to early development (Lopez et al. 2011).

Finding ways to ameliorate neurobiological alterations caused by stress in early development may help prevent future excessive alcohol consumption. The purpose of the current study is to evaluate the effect of exposure to an enriched environment in this previously established model of isolation during adolescence that induces high alcohol self-administration (Lopez et al. 2011). Rodent housing environments have historically been designed to provide for the animal's physiological needs and standardized for the benefit of the science conducted. As the field of laboratory animal medicine evolves, focus has been directed towards providing enrichment that would allow mice to perform natural behavior patterns. The phrase "environmental enrichment" is a concept that encompasses the ability to expand an animal's option for species-specific behavioral expression with the result being a positive effect on the both physiological and psychological well being (Bond et al. 2002; Young 2003). The mouse's species-specific overarching behavioral repertoire includes burrowing and nest building (Bond et al. 2002), and the bulk of the literature using preference testing to evaluate the impact of environmental enrichment in mice has shown

that mice prefer nesting material over any other form of enrichment material (Heizmann et al. 1998; Olsson and Dahlborn, 2002). Important to the present study, prior evidence indicates that cage enrichments have long lasting behavioral effects in mice that are opposite of those induced by chronic isolation (Meijer et al. 2007; Van de Weerd, 2002). Therefore, the hypothesis behind this study is that providing an enriched environment to mice housed in isolation during adolescence would reduce stress and anxiety and decrease the risk for elevated voluntary ethanol intake.

Methods and Materials

Study Design

The study is based on a factorial design with housing condition (single or group housed), enrichment (enriched environment or not) and sex (male and female) as main factors. A timeline for the study is presented in Figure 1. Among rodents, adolescence is broadly defined as the period between weaning and 60 days of age (38; 39). At weaning (Day 21), mice were randomly assigned to one of the four housing conditions (group-housed with or without enriched environment, or single-housed with or without enriched environment). At postnatal day 60 (PD60), half of the mice representative of the each housing situation and sex were evaluated for basal levels of corticosterone (described below) while the other half were tested for anxiety-like behavior (described below). After these evaluations, all mice were single-housed without enrichment to be evaluated for ethanol intake as described below. After three weeks of voluntary ethanol intake testing, and following three days of ethanol withdrawal, mice were evaluated again for behavioral indexes of anxiety-like behavior and physiological levels of stress. In this case, mice that were evaluated for corticosterone levels were tested for anxiety-like behavior and vice versa. This precaution was taken to avoid the effect of experience with the apparatus on anxiety levels as well as to avoid the effect of experience with the blood extraction procedure on corticosterone levels.

Specific methods

All procedures were approved by the Institutional Animal Care and Use Committee and followed the NIH Guide for the Care and Use of Laboratory Animals (8th edition, National Research Council, 2011).

Subjects—A total of 187 mice were used to collect data for this study (n=8–12/group). Adult specific pathogen free male and female C57BL/6J mice purchased from Jackson Laboratories (Bar Harbor, ME) were used in the study. Breeding pairs were housed in the standard caging described below with the same type of enrichment as described below. Cages were periodically checked for pregnancies. Nesting enrichment was provided until weaning. The day that pups were delivered was considered postnatal day 0 (PD0). All animals used in these experiments were weaned at PD 21. At weaning, mice were weighed and housed in groups (4 per cage) according to sex or individually housed. Precautions were taken to avoid having more than two mice coming from the same litter in a given experimental group (Spear and File, 1996).

Housing conditions—Single or group-housed mice (4 mice/cage) were housed in standard static filter top mouse shoebox caging (32.50 cm × 18.10 cm × 14.20 cm) with regular corncob bedding. Mice in the enriched environment situation were provided with two cotton nestlets. Mice had free access to food (Harland Teklad, Madison, WI) and water throughout all phases of the experiments. Temperature, humidity were kept within Guide recommended standards with a 12-h light/dark cycle (lights off at 1400 h) followed those used in our institution's AAALAC-accredited facility. Animal care facility staff provided standard care (cages changed once weekly) and mice were weighed weekly throughout the study.

Ethanol intake—A home-cage limited access paradigm was used in this study. Mice were given daily access to ethanol for 2-h in the home cage, beginning at 0.5 h prior to the start of the dark cycle (1330 h). This procedure takes advantage of the natural occurrence of high food and fluid intake that occurs at the beginning of the dark phase of the circadian cycle. Standard water bottles were removed and replaced with 15-ml graduated tubes containing an ethanol solution and a bottle containing tap water as an alternative fluid. The ethanol solution used was 15% (v/v). The ethanol concentration (15% v/v) was chosen because it represents a relatively high concentration that C57BL/6J mice readily consume in a relatively short period of time. The session duration of two hours was chosen because total amount of ethanol consumed (g/kg) within this time frame is tightly correlated with blood ethanol levels registered immediately after the access period (Becker and Lopez, 2004). At the end of the daily 2-h access periods, ethanol and water graduated tubes were removed and standard water bottles returned to the home cages. The sipper tube of the graduated tubes was identical to the sipper used for their regular water bottle. The position of the ethanol; and water tubes was alternated on a daily basis to avoid side preference. No side preference was observed in the study mainly because mice drank mostly from the ethanol tube during this limited access period. Body weights were recorded weekly and ethanol and water intake was measured daily (to the nearest 0.1 ml). Solutions were presented at room temperature and prepared fresh each day by mixing ethanol (95% ethanol) with deionized water to arrive at appropriate ethanol concentration (v/v). Mice were neither food nor water deprived at any time throughout the study. Ethanol intake in g/kg was served as the dependent variable.

Corticosterone assay—All blood samples were obtained at the beginning of the dark cycle (1400 h) to coincide with the timing of ethanol intake as well as minimize the impact of circadian cycle variations on this dependent variable. Blood was obtained from the retro orbital vein by a technician highly trained in this procedure. Based on our experience, this collection method does not induce immediate raise in corticosterone levels and provides enough blood for the assay (40µl). Blood was centrifuged and plasma was separated for the assay. Corticosterone plasma levels were determined by RIA (Weinberg and Bezio, 1987). Briefly, [3H] corticosterone (Perkin-Elmer) was incubated with an antibody (MP Biomedicals), and this complex removed using a charcoal-dextran solution. Free [3H]corticosterone was measured by liquid scintillation spectrometry. Samples were assayed in duplicate along with a standard curve and internal controls. Corticosterone levels are expressed as µg/dl of plasma.

Light/Dark box test—The behavioral procedure used exploits the natural tendency for rodents to avoid open and/or brightly lit spaces. This particular task has been shown to be sensitive to anxiety-like behaviors in mice (Bourin and Hascoet, 2003). Testing was done at the beginning of the dark cycle (1400 h). This time was selected to coincide with the time mice will be drinking ethanol. Mice were placed in sound attenuated chambers equipped with activity monitors designed for mice (Med-Associates; St. Albans, VT). The monitor floor is 28 cm × 28 cm and has three arrays of 16 infrared beams for automatic recording of activity, as well as enabling separate tracking and analysis of time spent on each side of the apparatus. An insert was placed in the arena to separate the monitor into light and dark compartments. Mice were tested for 15 minutes and testing began by placing the mouse in the light side of the apparatus. Time spent in the dark side of the apparatus, as well as the number of transitions between sides were recorded using software provided by the manufacturer (Med-Associates; St. Albans, VT).

Data Analysis—The main dependent variables for body weight (grams), corticosterone levels ($\mu\text{g/dl}$), time spent in the dark side of the light/dark test monitor (seconds) and ethanol intake (g/kg) were analyzed with a full factorial ANOVA model in which housing condition (single or group housed), enrichment (enriched environment or not) and sex (male and female) served as main factors (Statistica, StatSoft, Inc. Tulsa, OK). Ethanol intake analyses included days as repeated measure factor to evaluate possible daily variations on ethanol intake. Similarly, time was included as another factor in the analyses of corticosterone levels and anxiety-related dependent variables to evaluate possible differences in these variables recorded before versus after the ethanol intake-testing period. Whenever the ANOVA indicated a significant main effect of a factor or interaction between factors, post-hoc comparisons were conducted based on the error term of the effect or interaction with Bonferroni corrections to avoid Type I error. Significance level for all tests and post-hoc comparison were set a $p < 0.05$.

Results

Body weight

Analyses of body weight (grams) at the beginning of the ethanol intake test indicated only a significant effect of sex [$F(1,179)=477.93$, $p < 0.001$]. As expected, females were significantly lighter than males. Females weighed 19.56 ± 0.23 grams and males 24.56 ± 0.38 grams (values are mean $\pm$ SEM). None of the other factors under analysis reached statistical significance or interacted with the other factors of the study. This indicates that chronic housing conditions and enriched environment during early development do not affect body weight of male or female mice.

Basal corticosterone levels

Analysis of corticosterone levels before and after the ethanol intake test period indicated a significant main effect of sex [$F(1,165)=53.66$, $p < 0.01$] because females showed overall higher corticosterone levels. The ANOVA also indicated a significant main effect of time [$F(1,165)=66.60$, $p < 0.01$] because corticosterone levels across all conditions were higher at the end of the ethanol intake period compared to values observed before the start of ethanol

intake test (Figure 2). The ANOVA also indicated significant interaction between time and housing conditions [$F(1,165)=9.40$, $p<0.01$], because corticosterone levels after the ethanol intake test were higher in mice that were group-housed during adolescence (collapsed across sex) (Figure 3A). There was also a significant interaction between time and enriched environment [$F(1,165)=4.54$, $p<0.05$] because corticosterone levels after the ethanol intake period were higher in mice that did not have enriched environment compared to the other groups (collapsed across sex) (Figure 3B).

Light-Dark box test

The ANOVA indicated an overall main effect of test order [$F(1,152)=174.23$, $p<0.01$] because mice spent more time in the black section when tested before the ethanol intake testing period began. There was also a main effect of sex [$F(1,152)=13.16$, $p<0.01$] because males showed more anxiety-like behavior (more time in the dark side of the apparatus) than females. There was also a significant interaction between time of testing and housing conditions [$F(1,152)=10.36$, $p<0.01$] because group-housed mice showed more anxiety-like behavior than single-housed mice when tested before the ethanol intake-testing period (Figure 4). The number of transitions between sides was not affected by any of the factors under analysis (data not shown).

Voluntary ethanol intake

Regarding ethanol intake, the same factors were included in the analyses with the addition of days as within factor. Overall, females drank more than males [$F(1,179)=80.30$, $p<0.001$]. The ANOVA also reported a significant main effect of housing conditions [$F(1,179)=12.07$, $p<0.01$], and days [$F(14,2506)=20.09$, $p<0.001$] and significant interactions between sex and days [$F(14,2506)=2.05$, $p<0.05$], housing conditions and days [$F(14,2506)=2.29$, $p<0.01$] and enrichment and days [$F(14,2506)=2.13$, $p<0.01$]. Importantly, the ANOVA indicated a significant interaction of all 4 factors under analyses [$F(14,2506)=2.82$, $p<0.001$]. To further analyze the results of the overall ANOVA, separate analyses were conducted for each sex. These analyses confirmed the three-way interaction of housing condition, enrichment and days [$F(14,1302)=3.12$, and $F(14,1204)=2.05$; both $p<0.05$ for males and females, respectively]. Post-hoc comparisons indicated that chronic isolation without enrichment during adolescence induced higher drinking in both males and females compared to group-housed without enrichment. This effect was more pronounced during the first five days of testing. Figure 5, shows voluntary ethanol intake levels for each group during the first five days of the ethanol intake test where this effect was observed. This result confirms that the effect of chronic isolation on voluntary ethanol intake is transient as indicated in previous studies (Lopez et al. 2011). These results also showed that enriched environment counteracted the effect of chronic social isolation on alcohol intake. Mice chronically housed in isolation with enrichment did not drink more ethanol than control mice housed in groups with or without enrichment. As indicated above, and as it has been published (Becker and Lopez, 2004), mice drink almost exclusively from the ethanol bottle in this limited access protocol. Therefore, there was no effect of any of the factors under analyses on the voluntary intake of water or total fluid intake (data not shown).

Discussion

Many animal models have been used to study the impact of stress during early development on future ethanol intake (Becker et al. 2011; Sillaber and Henniger, 2004). Among rodents, adolescence is broadly defined as the period between weaning and 60 days of age (Spear 2000, 2004). In rodents, chronic stress during early developmental periods induces many behavioral changes, including altered anxiety responses, locomotor activity, and aggressive behaviors (Hefner and Holmes, 2007; Spear 2000, 2004). Social isolation during early development has been employed 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 (Gariépy et al. 1995; Guo et al. 2004; Zhu et al. 2006). This chronic stress situation has long-lasting effects on various behavioral and neural functions (Lapiz et al. 2003; Weiss et al. 2004). Importantly, chronic social isolation early in ontogeny can result in higher ethanol self-administration in adulthood in rats (Ehlers et al. 2007; Hall et al. 1998b; Juarez and Vazquez Cortes, 2003; McCool and Chappell, 2009) and mice (Advani et al. 2007; Lopez et al. 2011; Yanai and Ginsburg, 1976).

It has been well established that environmental enrichment induces biochemical and structural changes in the cortex and other brain regions of rodents and that these changes may account for the long-term impact of enrichments, as rodents enriched early in life perform better in learning and memory tasks as they age. Rampon et al. (2000) demonstrated that enrichment actually causes a significant change in the expression of genes whose products are involved in neuronal structure, plasticity and neurotransmission. Others have demonstrated that enrichment reduces reactivity to drugs of abuse (El Rawas et al. 2009; Stairs and Bardo, 2009). It is important to note that the enrichment prototypes used in the field of neurosciences are somewhat different than those used in laboratory animal science. The enrichment provided in neuroscience studies is more varied and complex than the simple addition of nesting material. Neuroscience enrichment items typically include: various toys, shelters, exercise wheels as well as nesting materials and the enrichment devices are often changed or used for focused time periods as neuronal plasticity is being evaluated. In addition social group and cage sizes may be varied (Sherwin and Olsson, 2004; van Praag et al. 2000; Zhu et al. 2006).

This study was designed to analyze if nesting material alone would enrich the environment sufficiently to alter the effect of chronic social isolation during early development on voluntary ethanol intake. In addition, this study evaluated whether chronic isolation with or without access to nesting material has an impact on stress/anxiety measures that could modulate voluntary ethanol consumption. Results showed that (regardless of sex) single-housed mice have a significant increase in voluntary ethanol intake, which can be reversed by providing nesting material (compared to the group-housed, non-enriched control group) and that providing this simple form of cage enrichment during a critical developmental period can significantly modulate voluntary ethanol intake later in life. In the current study we have demonstrated that C57BL/6J male and female mice will consume a substantial and stable amount of ethanol that should yield physiologically relevant blood ethanol levels immediately following the two-hour access session (Becker and Lopez, 2004). Moreover, the results obtained in this experiment replicate those of our previous studies (Lopez et al.

2011), since chronic isolation during adolescence induces an increase in voluntary ethanol intake. This effect was comparable to the effect of a chronic variable stress experience during adolescence (Lopez et al. 2011). The present study not only replicated the effect of chronic isolation on voluntary ethanol intake but it also indicated an important effect of providing an enriched environment. In this case, enrichment was able to counteract the effect of chronic isolation, since isolated mice housed in an enriched environment did not drink more than control mice that were socially housed (group housed).

Unlike previous studies (Tsai et al. 2002; Van de Weerd et al. 2002), we did not find that either the social housing condition or the provision of nesting material had an impact on body weight; however, the design of our study differed from those previous as it evaluated the impact during a phase of early development whereas other studies used adult mice. In addition, the length of time the animals were housed with the enrichment material was less than in previous studies (6 vs. 11 weeks) (Van de Weerd et al. 2002). Gaskill et al. (2012) demonstrated no change in body weight in relation to nesting enrichment, but did note that thermal distress, which could be a confounding variable in this study, could occur when less than 6 grams (compared to 0,2,4 grams) of nesting material is provided. The weight of the nesting material provided in this study was 5 grams in its compact form, a quantity that may/may not have alleviated thermal distress (Gaskill et al. 2012).

We also found no impact of enrichment on basal corticosterone levels. It is possible that this lack of effect of housing conditions on basal corticosterone levels could be linked to age-related factors that influence the physiological impact of housing on corticosterone levels. An important factor to consider as well is, that in the present study, only basal levels of corticosterone were measured. Mice were not challenged with a stressful event before measuring corticosterone levels, and HPA axis activation was not assessed. It would be very important to evaluate whether possible differences in HPA axis activation due to chronic housing conditions during adolescence could modulate ethanol consumption as ethanol itself has known activating effects on the HPA axis (Rivier 2000; Wand 2000).

There was, however, a significant interaction between time of testing and housing conditions because corticosterone levels were higher in mice that were housed without enriched environment after the three weeks of ethanol intake testing. It has been previously shown that nesting material can both decrease or not alter corticosterone levels. Van de Weerd et al. (1997) showed that nesting materials used as environmental enrichment did not affect corticosterone levels of group-housed mice, but noted a much greater variability in the corticosterone levels of mice housed in cages enriched with nesting material. They hypothesized that this might be due to an influence of enrichment on mouse social interactions. Providing nesting material may actually create greater stress in social situations as the mice may try to defend the enrichment resource from cage mates. However, the interaction between enriched housing conditions and voluntary ethanol intake on corticosterone levels have not been evaluated. This study did not challenge this variable and more research on the response of HPA axis to stress, and its relation to ethanol intake and mouse social paradigms would need to be undertaken.

Providing nesting material had no significant impact on the measure of anxiety-like behavior used; however, we did find a main effect of test order for the light/dark test because mice spent more time in the dark section when tested before the ethanol intake testing period began. There was also a main effect of sex in which males showed more anxiety-like behavior (more time in the dark side of the apparatus) than females. Unexpectedly, group-housed mice showed more anxiety-like behavior than single-housed mice when tested before the ethanol intake-testing period, but consumed less ethanol than single-housed mice throughout the duration of the study. This result was unexpected since previous studies have shown a different relationship between chronic housing effects on anxiety-like behavior and ethanol intake measures. Most studies have shown that chronic isolation induces higher levels of ethanol self-administration but also higher levels of anxiety (Becker et al. 2011; McCool and Chappell, 2009). In the study presented here, mice that drank the most showed lower levels of anxiety-like behavior. Previous studies conducted with male and female mice (Advani et al. 2007; Lopez et al. 2011; Yanai and Ginsburg, 1976) showed that chronic isolation induced higher levels of ethanol intake. However, none of these studies evaluated changes in anxiety levels after chronic isolation or the impact of anxiety on voluntary ethanol intake. Therefore, this issue merits further investigation. Also, a limitation of the present study is that anxiety was measured only with one task. The light/dark box is a very reliable test to measure anxiety-like behavior (Bourin and Hascoet, 2003); however, it would be better to evaluate changes in the anxiety trait of mice that were chronically housed in isolation with or without enrichment with a complementary battery of anxiety tests.

Taken together, the results presented here indicate that chronic social isolation during adolescence induces higher levels of voluntary ethanol intake in mice. This effect is evident during the initiation of ethanol intake during adulthood. As reported in previous studies, the effect is transient, and in this case was not related to the anxiety-like behavior level displayed by the subjects or their basal level of corticosterone. Importantly, this study also showed that providing enriched environment to socially isolated adolescent mice was sufficient to counteract the effect of chronic social isolation in regards to voluntary ethanol intake. This represents a new opportunity of research regarding the impact of social and environmental conditions during adolescence that would impact future ethanol intake.

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Highlights

Social isolation during adolescences promotes alcohol intake

Social isolation promotes changes in anxiety

Enrich environment reverses the effect of isolation on ethanol intake

Figure 1.
Timeline of the different stages of the study

Before ethanol testing

After ethanol testing

Figure 2.

Plasma corticosterone concentrations (µg/dl) registered in male and female mice in all four housing conditions either before (top) or after (bottom) the voluntary ethanol intake test period. Values are mean $\pm$ SEM.

Figure 3.

Plasma corticosterone concentrations (µg/dl) registered before or after the ethanol intake testing in **A**) single or group housed mice (collapsed across sex and enrichment conditions) and **B**) No Enriched or Enriched housing conditions (collapsed across sex and group or single housing conditions). Values are mean $\pm$ SEM.

* indicates a significant difference from the Before group.

Before ethanol testing

After ethanol testing

Figure 4.

Anxiety response measured as time in the dark side of the apparatus (seconds) from male and female mice in all four housing conditions evaluated either before (top) or after (bottom) the voluntary ethanol intake test period. Values are mean $\pm$ SEM. * indicates a significant main effect of group vs. single housing.

Figure 5.

Ethanol intake levels (g/kg) for male (top) and female (bottom) in the four different housing conditions registered at the beginning of the ethanol intake test period. Data is averaged over the first 5 days of testing. Values are mean $\pm$ SEM.

* indicates a significant difference from Group No enriched group.