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Affective Behavior in Withdrawal Seizure-Prone and Withdrawal Seizure-Resistant Mice during Long-Term Alcohol Abstinence

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Abstract

Background: While the acute alcohol withdrawal syndrome has been well-characterized in both human clinical studies and in experimental animals, much less is known regarding long-term affective disturbances that can sometimes persist during protracted abstinence. Nevertheless, since relapse often occurs long after acute detoxification, and may be predicted by persistent affective disruption, a better understanding of the long-term behavioral consequences of prior alcohol dependence may lead to improved strategies for relapse prevention.

Methods: Male and female Withdrawal Seizure-Prone and Withdrawal Seizure-Resistant mice from the second selection replicate (WSP-2, WSR-2) were exposed to a 10-day chronic-intermittent ethanol vapor protocol (CIE) or plain air, and then tested repeatedly on the sucrose preference test (SPT), marble burying test (MBT), and the light-dark box test (LDT) over 7 weeks of (forced) abstinence.

Results: While WSP and WSR mice differed significantly on tests of anxiety-like behavior (LDT, MBT), we found little evidence for long-term affective disruption following CIE in either line. The major exception was in the LDT, in that WSP but not WSR mice displayed longer latencies to enter the light compartment following CIE relative to air-controls.

Conclusions: Selective breeding for acute withdrawal severity has resulted in differences in anxiety-like behavior between WSP and WSR mice. In contrast, however, genes contributing to the severity of acute withdrawal convulsions appear to have little overlap with those predisposing to affective disruption during long-term abstinence.

Keywords

CIE; affective behavior; protracted abstinence; WSP; WSR

1. Introduction

The alcohol withdrawal syndrome is a potentially life-threatening state that is caused by termination of alcohol consumption in individuals who have consistently engaged in chronic heavy drinking. Symptom onset is typically within eight hours of initial abstinence, and can include irritability, nausea, vomiting, insomnia, tremor, hyperalgesia, hyperthermia, tachycardia, anxiety, hallucinations, delusions, and tonic-clonic seizures (American Psychological Association, 2013; Atilia et al., 2018). Although these acute symptoms can be effectively managed with benzodiazepines (Mayo-Smith, 1997), such treatments do not significantly affect the likelihood of eventual relapse (Malcolm et al., 2002; Askgaard et al., 2016). Much less is known concerning the longer-term affective-behavioral disruptions that may emerge weeks to months after initial detoxification, and often persist across even prolonged periods of abstinence. In affected individuals, persistent anxiety and depressive symptoms may contribute to an increased risk of relapse long after acute detoxification (Malcolm, 2003; Heilig, Egli, Crabbe, & Becker, 2010). Additionally, benzodiazepines do not significantly mitigate emerging affective disturbances when administered several weeks into abstinence (Gallant, Bishop, Guerrero-Figueroa, Selby, & Phillips, 1969).

Animal models have been very useful in identifying phenotypic and genotypic factors associated with acute alcohol (ethanol) withdrawal and in characterizing behavioral disruptions during long-term (forced) abstinence. Since laboratory animals rarely consume a sufficient volume of ethanol in free-choice experiments to produce dependence, several methods have been developed to induce experimental ethanol dependence in rats and/or mice (Lovinger & Crabbe, 2005; Becker & Ron, 2014; Holleran & Winder, 2017). One method that has been widely employed in both species involves the forced inhalation of volatilized ethanol vapor (Becker, 2013). While a wide variety of protocols have been used, ethanol vapor exposure is capable of quickly and effectively inducing dependence, which can be demonstrated in mice by an elevation in the intensity of handling-induced convulsions (HICs) upon withdrawal (Goldstein & Pal, 1971; Goldstein, 1972; Becker & Hale, 1993; Becker, Diaz-Granados, & Weathersby, 1997). In addition, several experiments have revealed increases in anxiety- and/or depressive-like behavior in rats (Valdez et al., 2002; Zhao, Weiss, & Zorrilla, 2007; Walker et al., 2010) and mice (Logan, McCulley, Seggio, & Rosenwasser, 2012; Sidhu, Kreifeldt, & Contet, 2018) weeks after the termination of vapor exposure. To date, however, little is known regarding possible linkages between the acute and long-term sequelae of ethanol dependence (Heilig et al., 2010).

Studies by Crabbe and colleagues have revealed considerable genetic variance among inbred mouse strains in HIC scores following both continuous and chronic-intermittent ethanol vapor exposure (CIE) (Metten & Crabbe, 2005; Metten, Sorensen, Cameron, Yu, & Crabbe, 2010). Further, the Crabbe laboratory has used bidirectional selective breeding of genetically heterogeneous mice (HS/Ibg) from an 8-way cross of inbred strains to develop multiple stable lines of Withdrawal Seizure-Prone (WSP) and Withdrawal Seizure-Resistant (WSR) mice displaying high and low HIC scores, respectively, following 72-hour ethanol vapor exposure (Crabbe, Kosobud, & Young, 1983; Crabbe, Kosobud, Young, Tam, & McSwigan, 1985). Interestingly, these selected lines have been tested for differences on various other

ethanol-related phenotypes, with mixed results. For example, although WSP and WSR mice show differences in the Drinking-in-the-Dark paradigm (Crabbe et al., 2013) and exhibit differentially altered prefrontal cortical gene expression during forced abstinence (Hashimoto, Forquer, Tanchuck, Finn, & Wiren, 2011; Wilhelm, Hashimoto, Roberts, Sonmez, & Wiren, 2014; Hashimoto, Gavin, Wiren, Crabbe, & Guizzetti, 2017), they do not significantly differ in free-choice ethanol drinking, operant ethanol self-administration, or expression of the alcohol deprivation effect (ADE; Ford et al., 2011; Crabbe et al., 2013; Rosenwasser, Fixaris, Crabbe, Brooks, & Ascheid, 2013). Currently, however, nothing is known regarding possible persistent withdrawal-associated affective disruptions in these selected lines.

In the current experiment, male and female WSP and WSR mice from the second selection replicate (WSP-2, WSR-2) were exposed either to a 10-day CIE protocol or to plain room air in inhalation chambers, and subsequently administered repeated well-established tests of affective behavior (sucrose preference test, SPT; marble burying test, MBT; light-dark box test, LDT) over the course of 7 post-Tx weeks. We hypothesized that post-dependent WSP mice would be more likely than WSR mice to display persistent affective disturbances during forced abstinence. Such a result would imply that overlapping sets of genes contribute to both acute (e.g., increased seizure susceptibility) and long-term (e.g., increased anxiety- or depressive-like behavior) consequences of dependence and withdrawal.

2. Methods

2.1 Animals

Male and female WSP-2 (M, $n = 30$; F, $n = 30$) and WSR-2 (M, $n = 19$; F, $n = 30$) mice were shipped to the University of Maine from breeding colonies maintained at the Portland VA Medical Center (Portland, OR). Mice arrived in the laboratory at approximately 6 weeks of age and were immediately group-housed by sex, line, and assigned treatment (5 per cage), in large mouse cages ($32 \times 20 \times 14$ cm). Animals were housed under a LD 12:12 lighting regimen with food (Prolab RMH 3000; LabDiet, St. Louis, MO) and tap water freely available throughout the experiment. All experimental procedures were approved by the University of Maine Institutional Animal Care and Use Committee (IACUC).

2.2 Procedures

Mouse housing cages were initially kept within larger inhalation chambers with the system fan turned on and circulating plain room air for 2 weeks of acclimation. The inhalation chambers consisted of large Plexiglas boxes ($60 \times 36 \times 60$ cm) constructed according to a design provided by Dr. Howard Becker (Medical University of South Carolina). After acclimation, animals were exposed to a 10-day CIE protocol (see below), while controls were handled identically, but exposed only to plain air. Following CIE or air treatment, animals were single-housed in standard mouse cages ($30 \times 18 \times 12$ cm) in a light-shielded and sound-attenuating metal cabinet equipped with a standard fluorescent bulb on each shelf. Animals underwent 5 total weeks of behavioral testing (see below) during a 7-week abstinence period, with all behavioral tests beginning at the onset of the dark phase. Upon termination of the CIE protocol, animals were tested for 4 consecutive weeks, followed by a

2-week rest period and a final test in week 7. For each test week, the order of behavioral tests was as follows: (1) sucrose preference test (SPT), (2) marble burying test (MBT), and (3) light-dark box test (LDT). 48 hours separated the SPT and MBT, while 72 hours separated the MBT and LDT. During consecutive weekly testing, 48 hours separated the final test of one week and the initial test of the successive week. This order was designed to minimize the effects of repeated administration, with larger gaps between potentially more invasive behavioral tests. Experimental and control groups consisted of 9–15 animals per group for each sex/line; *n*'s for each group are available in Table 1.

2.2.1 Chronic-Intermittent Ethanol Protocol—In the present work, a 10-day CIE protocol was employed in which ethanol vapor was delivered to the experimental chambers for 16 hours per day alternating with 8 hours of plain air for 10 consecutive days, with each vapor exposure period beginning at dark onset. Air-control animals were handled identically, but exposed only to plain air. Immediately prior to each vapor exposure period, CIE animals were administered a priming injection containing 1.6 g/kg ethanol and 68.1 mg/kg pyrazole HCl, an alcohol dehydrogenase inhibitor used to rapidly increase and stabilize BEC (Becker & Hale, 1993). Pyrazole was dissolved in 20% v/v ethanol solution and injected *i.p.* in a volume of 10 mL/kg. Air-control animals were administered an identical dose of pyrazole in 0.9% saline solution, but without ethanol, at the same injection volume. All animals were weighed prior to and halfway through the 10-day CIE cycle to ensure appropriate injection volumes, and to monitor possible CIE-induced changes in body weight (see below). Ethanol was vaporized using a pressurized pump to push air through a porous diffusing stone submerged in a 1.0-L bottle filled with 95% ethanol. A calibrated mixture of ethanol vapor and plain room air was continuously delivered during the daily 16-hour ethanol exposure periods, while plain air was delivered at other times. Plain air was always provided at a flow rate of 10 L/min throughout the experiment in order to ensure airflow adequate to meet the animals' respiratory requirements. Because prior studies had shown that metabolic tolerance to ethanol develops across multiple-day CIE protocols (Metten and Crabbe, 2005; Metten et al., 2010), we adopted a method from Metten et al. (2010) and gradually increased the flow of ethanol vapor from 1.3 to 2.4 L/min across the 10 days of CIE in an effort to produce stable blood ethanol levels. Unlike Metten et al. (2010), however, we only measured BECs at the end of the protocol, so we have no objective measure of the success of this maneuver. Chamber ethanol concentrations were measured on a daily basis by extracting 5.0-mL air samples from the exposure chambers through a rubber stopper using a 60-mL syringe, mixing the sample with 55 mL of ambient air, and injecting the diluted sample into a breathalyzer (Lifeloc FC-10; Wheat Ridge, CO). Breathalyzer readings were then compared to a standardized calibration curve to determine chamber ethanol concentrations. As intended, chamber ethanol concentrations progressively increased across days, ranging from 10 to 18 mg/L, typical for CIE studies with mice.

2.2.2 Measurement of Ethanol Concentrations in Tail Blood—BECs were measured in a subset of vapor-exposed mice immediately following the final ethanol vapor exposure period. A small (approximately 10 μ L) blood sample was collected from the tip of the tail of each mouse and centrifuged for 2 minutes to separate plasma from serum. BECs

were determined from 5 μ L plasma samples using an AM-1 alcohol analyzer (Analox Instruments; Lunenburg, MA).

2.2.3 Body Weights—Body weights were obtained in CIE and air-control animals at the beginning of the CIE protocol, on day-6 of the CIE, and at the termination of the final CIE cycle. The effects of CIE on body weight were evaluated by computing percent body weight change from the beginning to the end of the CIE protocol.

2.2.4 Sucrose Preference Test—Animals were offered two-bottle, free-choice access to a 0.75% sucrose solution and plain water for 3 hours. Pre- and post-measurements of bottle weight (g) were used to obtain overall intake. Sucrose preference was determined by dividing the volume of sucrose solution consumed by total fluid intake. Decreases in sucrose preference are generally interpreted as “anhedonic behavior” (Katz, 1982), an inability to derive pleasure from normally pleasurable stimuli, which is one of the defining symptoms when diagnosing Major Depression Disorder (APA, 2013).

2.2.5 Marble Burying Test—Animals were placed within a square cage ($30 \times 30 \times 15$ cm) containing flattened bedding material and 25 identical black marbles arranged in an evenly spaced 5×5 grid. Animals were permitted to freely-move about the cage for 7 minutes, while dim red light (<5 lux) from an overhead lamp provided sufficient illumination for video-recording. The total number of marbles buried within the bedding material were counted; only marbles with approximately 75% of their area hidden were considered “buried”. While marble burying is thought to reflect anxiety-like behavior, and is reduced by anxiolytic drug treatments (Borsini, Podhorna, & Marazziti, 2002), there is still some debate in the literature with regard to the exact psychological significance of this test (Londei, Valentini, & Leone, 1998; Thomas et al., 2009).

2.2.6 Light-Dark Box Test—Animals were placed in a two-compartment test chamber in which one compartment ($27 \times 17 \times 27$ cm) is kept darkened while the other ($27 \times 27 \times 27$ cm) is illuminated via an overhead lamp (~ 550 – 650 lux). The compartments are separated by a wall with a small central opening (6×6 cm) through which the mouse can easily shuttle between the two compartments. Animals were initially placed in the dark compartment and were permitted to freely-move about the apparatus for 6 minutes. Behavior in the environment was video-recorded and the following parameters were extracted: (1) percentage of time spent in the light compartment (2) latency to first entry to the light compartment, and (3) total transitions between compartments. Less time spent in, and longer latencies to initially enter, the light compartment, are interpreted as anxiety-like behavior. There is also pharmacological evidence that anxiolytic drugs increase the percentage of time an animal spends in the light compartment (Bourin & Hascoët, 2003). A square root transformation was applied to the latency data in order to combat skewness of the distribution while maintaining statistical power (Whelan, 2008). Due to software error, data from a single post-Tx test day were unavailable for a subset of animals’ (WSP, $n = 20$; WSR, $n = 18$), and these animals were thus excluded from repeated-measures analyses of LDT measures.

2.2.7 Statistics—Initial body weight and percent body weight change were analyzed using 3-factor (treatment, line, sex) ANOVA, while BECs were analyzed in ethanol-exposed animals using 2-factor (line, sex) ANOVA. Behavioral data were initially analyzed using 4-factor (treatment, line, sex, post-Tx day) mixed-design analysis of variance (ANOVA). When significant effects involving sex were detected, this analysis was followed-up by conducting separate 3-factor ANOVAs (treatment, line, post-Tx day) for males and females, and data are presented separately by sex. In the absence of sex-related effects, data were collapsed across sex to increase power to detect significant effects of treatment and line. When significant main effects of post-Tx day were detected, Bonferroni-adjusted t-tests were used to make all possible pairwise comparisons among test days. When significant interactions involving post-Tx day were seen, these were followed-up by conducting separate ANOVAs for each individual test day including any relevant factors (treatment, line, and/or sex). Full ANOVA results (F , df , p , partial η^2) are given for the initial 4-factor analysis, but only p -values are given for follow-up tests.

Data are presented as means $\pm$ SEM, and effects were considered to be statistically significant when $p < .05$. Data analysis was performed using SPSS 23.0 (IBM Inc., Armonk, NY) and figures were generated using SigmaPlot 10.0 (Systat Software Inc., San Jose, CA).

3. Results

3.1 Body Weights and BECs

Prior to treatment, males weighed significantly more than females [$F(1, 101) = 236.55$, $p < .001$, partial $\eta^2 = .701$] and WSR animals weighed significantly more than WSP animals [$F(1, 101) = 22.40$, $p < .001$, partial $\eta^2 = .182$], but there were no significant differences between assigned treatment (Table 1). WSR animals gained significantly less weight than WSP animals over the course of treatment [$F(1, 101) = 6.61$, $p = .012$, partial $\eta^2 = .061$], and while there were no significant effects of sex or treatment, ethanol-exposed animals gained less weight than plain air-controls (1.44% vs. 3.44%). Lastly, all groups showed BECs above the threshold for intoxication, and while there were no significant effects of sex or line, females showed somewhat higher BECs than males (Table 2).

3.2 Sucrose Preference Test

ANOVA revealed a significant main effect of post-Tx day [$F(4, 404) = 11.57$, $p < .001$, partial $\eta^2 = .103$] and a significant treatment $\times$ post-Tx day interaction [$F(4, 404) = 2.72$, $p = .038$, partial $\eta^2 = .026$], but no significant effects involving line or sex (Fig. 1). Pairwise comparisons among post-Tx days showed that sucrose preference was significantly greater on post-Tx day 1 than post-Tx days 8, 15, and 43 (p 's $< .05$), but not post-Tx day 22, while separate 1-factor ANOVAs for each test day revealed that ethanol-exposed animals exhibited significantly lower sucrose preference than air-controls on post-Tx day 15 ($p = .006$), but not on any other test day.

3.3 Marble Burying Test

ANOVA revealed significant main effects of line [$F(1, 101) = 16.51$, $p < .001$, partial $\eta^2 = .140$], sex [$F(1, 101) = 13.96$, $p < .001$, partial $\eta^2 = .121$], and post-Tx day [$F(4, 252) = 8.37$,

$p < .001$, partial $\eta^2 = .077$], as well as a significant line x sex interaction [$F(1, 101) = 6.24$, $p = .014$, partial $\eta^2 = .058$], but there were no significant effects of ethanol treatment (Fig. 2). Pairwise comparisons among post-Tx days showed that total marbles buried was significantly lower on post-Tx day 17 than on post-Tx days 3, 10, and 45 (p 's $< .05$), but not on post-Tx day 24. Overall, males buried more marbles than females, and WSR animals buried more marbles than WSP animals, but the effect of line was significant only in females ($p < .001$).

3.4 Light-Dark Box Test

Percentage of time in light: ANOVA revealed significant main effects of line [$F(1, 63) = 136.96$, $p < .001$, partial $\eta^2 = .685$] and post-Tx day [$F(4, 252) = 4.40$, $p = .002$, partial $\eta^2 = .065$], but there were no effects of sex or treatment (Fig. 3). Pairwise comparisons among post-Tx days revealed that time spent in light was significantly lower on post-Tx day 6 than post-Tx days 13, 20, and 27 (p 's $< .05$), but not post-Tx day 48. Overall, WSP animals spent significantly less time in the light compartment than WSR animals.

Latency to enter light: ANOVA revealed significant main effects of line [$F(1, 63) = 18.44$, $p < .001$, partial $\eta^2 = .266$], treatment [$F(1, 63) = 6.52$, $p = .013$, partial $\eta^2 = .094$], and post-Tx day [$F(4, 252) = 37.95$, $p = .015$, partial $\eta^2 = .048$], but no effects of sex (Fig. 3). WSP and ethanol-exposed animals exhibited significantly longer latencies to enter the light compartment compared to WSR animals and air-controls, respectively, and pairwise tests showed that latency was significantly higher on post-Tx day 13 than post-Tx day 20 ($p = .047$) but not any other day. Despite the lack of a significant line x treatment interaction ($p = .054$), we conducted separate exploratory tests for treatment effects on each post-Tx day for each line; these tests showed significant treatment effects in WSP mice on post-Tx days 13 ($p = .008$) and 20 ($p = .018$), but no treatment effects on any day in WSR. Finally, we note that 40% of ethanol-exposed WSP mice failed to emerge from the dark compartment on at least one post-Tx day, compared to 5% of ethanol-exposed WSR, 15% of air exposed WSP, and 0% of air-exposed WSR.

Total transitions: ANOVA revealed significant main effects of treatment [$F(1, 63) = 6.11$, $p = .016$, partial $\eta^2 = .088$] and post-Tx day [$F(4, 252) = 5.87$, $p < .001$, partial $\eta^2 = .085$], as well as a significant line x treatment x post-Tx day interaction [$F(4, 252) = 2.52$, $p = .042$, partial $\eta^2 = .038$]. Overall, ethanol-exposed animals displayed significantly fewer total transitions than air-controls, while pairwise tests showed that total transitions were significantly lower on post-Tx day 13 than post-Tx days 20, 27, and 48 (p 's $< .05$), but not post-Tx day 6. To further explore the 3-way interaction between line, treatment and post-Tx day, separate line x treatment ANOVAs were conducted for each individual test day. A significant main effect of line was present on each test day (p 's $< .05$), except post-Tx day 20, as WSR animals displayed greater total transitions than WSP animals. A significant main effect of treatment was present on post-Tx days 13, 20, and 27 (p 's $< .05$), but not on post-Tx days 6 or 48, as ethanol-exposed animals displayed fewer total transitions than air-controls (Fig. 3). Nevertheless, a significant line by treatment interaction was not seen for any individual test day.

4. Discussion

Overall, this study detected significant test-dependent effects of line, CIE treatment, sex, and post-Tx day. WSP and WSR mice displayed significant differences in tests of anxiety-like behavior (MBT, LDT), but not depressive-like behavior (SPT), suggesting that selective breeding for acute ethanol withdrawal convulsion severity resulted in differential segregation of anxiety-related alleles in the two lines. Relative to air controls, CIE produced significant long-term changes in affective behavior only in the LDT, suggesting that different aspects of affective behavior may be expressed differentially during long-term abstinence. Of course, the major focus of this study was to uncover possible differences in affective behavior between post-dependent WSP and WSR mice that might be related to differences in their acute withdrawal severity. In this regard, we found suggestive evidence that WSP mice may exhibit greater and/or more persistent anxiety-like behavior in the LDT following CIE.

While WSP and WSR mice showed behavioral differences in both the LDT and MBT, these were apparently in opposite directions, consistent with suggestions that the LDT and MBT may assay different phenotypic aspects of anxiety. In the present study, WSP mice displayed more anxiety-like behavior than WSRs in the LDT, consistent with previous findings that WSP mice exhibit greater anxiety than WSRs in the elevated-plus maze (Gorin, Crabbe, Tanchuck, Long, & Finn, 2005) and canopy stretched-attend-posture (Atkins, Rustay, & Crabbe, 2000) tests. Conversely, however, WSR (females) exhibited greater anxiety-like behavior than WSPs on the MBT, possibly related to their slightly higher BECs relative to males. Although marble burying is reduced by anxiolytic drug treatments (Borsini et al., 2002), responsiveness to such pharmaceutical treatment does not automatically validate a model for anxiety-like behavior (Njung'e & Handley, 1991), and there is continuing debate within the literature regarding the psychological significance of the MBT (Londei et al., 1998; Thomas et al., 2009).

Final BECs achieved in this study averaged ~110 mg/dL, somewhat lower than some previous CIE studies. Thus, Metten and Crabbe (2005) reported strain mean BECs ranging between 130 and 208 mg/dL across a large panel of inbred strains, measured after 72 hours of continuous ethanol vapor exposure. In an otherwise similar study but employing a 3-day intermittent vapor protocol, Metten et al. (2010) found that BECs were generally lower after intermittent than after continuous exposure, and also quite variable across strains and individuals after similar exposure protocols. Perhaps most importantly for the present study, Metten et al. (2010) also found that BECs between about 100 and 130 mg/dL were still sufficient to result in elevated convulsion scores during acute withdrawal.

Thus, while it is possible that more extensive effects of CIE may have been seen with higher BECs, the present study noted significant effects of CIE treatment in the LDT and – marginally – in the SPT. In the SPT, ethanol-exposed animals showed lower levels of sucrose preference than air controls only on a single post-Tx day (day 15), suggesting that any effects of CIE in this test were transient. It should also be noted that, under the specific conditions of this experiment (3-hour test, sucrose concentration of 0.75%), an absolute preference (i.e., preference ratio > 50%) for sucrose over plain water was rarely observed, and mainly only in the first post-TX test day. Sucrose preference is known to vary

dramatically across mouse strains, and it is possible that WSP and WSR mice are less sensitive to sucrose than other lines. Thus, while Pothian et al. (2004) reported preferences between 75–94% for a 1% sucrose concentration in a panel of 11 inbred strains (1-day test), Crabbe et al. (2013) reported preferences of about 60% at a sucrose concentration of 1.7% in male and female WSP and WSR mice (4-day test). In any case, given the relatively low sucrose preferences observed in the present study, any possible implications of these data for depression-like anhedonia should be approached with caution.

In the LDT, ethanol-exposed mice showed longer latencies to enter the light compartment and fewer total transitions between compartments than air controls across the entire duration of post-Tx testing, suggesting that CIE induced a rapid and long-lasting increase in behavioral anxiety. These observations are consistent with previous work showing that forced abstinence from ethanol vapor can elicit anxiety-like behaviors in diverse mouse populations (Finn, Gallaher, & Crabbe, 2000; Kliethermes, Cronise, & Crabbe, 2004; McCool & Chappell, 2015; Metten et al., 2017; Sidhu et al., 2018).

While the number of transitions is sometimes treated as a measure of locomotor activity and as a potential confound in the assessment of anxiety-like behavior in tests such as the LDT that involve animals' voluntary movement between "safe" and "threatening" environments, evidence suggests that general locomotion and anxiety-related behaviors are inherently negatively correlated and thus difficult to dissociate (Kliethermes, 2005; Milner & Crabbe, 2008). In contrast to the effects of CIE on latencies and total transitions, ethanol treatment failed to affect the percentage of test time spent in the light compartment. Thus, in the absence of CIE effects, significant differences among post-Tx day seen for this measure probably reflect non-systematic variation across test days. Perhaps the most important – if somewhat tentative – finding of this study, however, is that persistent effects of CIE on at least some aspects of anxiety-like behavior in the LDT were seen mainly in WSP mice. While this finding will require further replication and confirmation, it suggests that there may be a genetic linkage between acute, physiological withdrawal severity and affective disruption during long-term abstinence.

Our results generally support the hypothesis that affective consequences of ethanol withdrawal may significantly outlive the immediate somatic signs of withdrawal. Specifically, these findings confirm prior studies indicating that selective breeding for acute ethanol withdrawal severity results in the emergence of line differences in trait-like anxiety, and also provide evidence for persistent affective disruption in the LDT during long-term forced abstinence in mice. Finally, we saw tentative evidence linking genetic differences in acute withdrawal severity to persistent anxiety-like behavior in abstinence. Further work will be required to confirm and expand these initial findings.

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Figure 1.

Mean ($\pm$ SEM) sucrose preference following 10-day chronic-intermittent ethanol (Ethanol) and control (Air) treatments in Withdrawal Seizure-Prone (WSP) and Withdrawal Seizure-Resistant (WSR) mice.

Figure 2.

Mean ($\pm$ SEM) number of marbles buried following 10-day chronic-intermittent ethanol (Ethanol) and control (Air) treatments in female (top) and male (bottom) Withdrawal Seizure-Prone (WSP) and Withdrawal Seizure-Resistant (WSR) mice.

Figure 3.

Mean ($\pm$ SEM) percent of time spent in light (top), latency of first transition to light (middle), and transitions (bottom) following 10-day chronic-intermittent ethanol (Ethanol) and control (Air) treatments in Withdrawal Seizure-Prone (WSP) and Withdrawal Seizure-Resistant (WSR) mice.

Table 1.

Mean ($\pm$ SEM) body weight (BW) in male (M) and female (F) WSP and WSR mice immediately prior to and following CIE or air-control (CON) treatment and percent change in body weight during treatment.

Line	Sex	Treatment	<i>n</i>	Pre-Tx BW (g)	Post-Tx BW (g)	% BW change
WSP	F	CON	15	20.6 $\pm$ 0.45	21.9 $\pm$ 0.60	+6.10 $\pm$ 1.59
WSP	F	CIE	15	21.2 $\pm$ 0.47	22.0 $\pm$ 0.58	+3.70 $\pm$ 1.70
WSP	M	CON	15	26.2 $\pm$ 0.53	26.9 $\pm$ 0.42	+3.11 $\pm$ 1.29
WSP	M	CIE	15	26.4 $\pm$ 0.45	27.1 $\pm$ 0.44	+2.94 $\pm$ 2.10
WSR	F	CON	15	22.0 $\pm$ 0.36	22.4 $\pm$ 0.44	+1.93 $\pm$ 0.78
WSR	F	CIE	15	22.3 $\pm$ 0.50	21.6 $\pm$ 0.50	-2.73 $\pm$ 2.11
WSR	M	CON	9	28.6 $\pm$ 1.02	29.3 $\pm$ 1.00	+2.64 $\pm$ 0.67
WSR	M	CIE	10	28.9 $\pm$ 0.80	29.5 $\pm$ 1.00	+1.85 $\pm$ 1.41

CIE, chronic-intermittent ethanol; WSP, Withdrawal Seizure-Prone; WSR, Withdrawal Seizure-Resistant.

Table 2.

Mean ($\pm$ SEM) BECs for ethanol-exposed male and female WSP and WSR mice immediately following the 10-day CIE protocol.

Line	Sex	Treatment	<i>n</i>	BEC (mg/dL)
WSP	F	CIE	11	129.27 $\pm$ 24.86
WSP	M	CIE	15	95.15 $\pm$ 17.06
WSR	F	CIE	15	122.29 $\pm$ 19.80
WSR	M	CIE	8	90.85 $\pm$ 22.43

BEC, blood ethanol concentrations; other abbreviations as in Table 1.