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ASSESSMENT OF DEPRESSION-LIKE BEHAVIOR AND ANHEDONIA AFTER REPEATED CYCLES OF BINGE-LIKE ETHANOL DRINKING IN MALE C57BL/6J MICE

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Abstract

Psychological depression is frequently linked to alcohol abuse and even serves as key indicators of an alcohol use disorder (AUD). This relationship is supported by preclinical findings in which depression-like phenotypes develop in animals exposed to chronic intermittent ethanol vapor, a common preclinical model of alcohol dependence. However, the emergence of these maladaptive phenotypes following repeated binge-like ethanol drinking remains relatively unexplored. The purpose of this study was to evaluate depression-like behaviors associated with binge-like consumption in mice. Using the drinking-in-the-dark (DID) paradigm, we examined the impact of multiple binge-like cycles (1, 3, or 6) on depression-like behaviors in the forced swim test (FST) and sucrose preference as a test for anhedonia. We also assessed the effect of repeated binge cycles on the consumption of bitter and sweet tastants over a range of concentrations. Results indicated that binge-like ethanol drinking did not lead to depression-like behavior as repeated cycles of DID did not alter sucrose consumption or preference nor did it impact time spent immobile during the FST. Animals that experienced six cycles of DID showed increased quinine consumption and increased quinine preference, which may be indicative of an escalated preference for tastants that resemble the gustatory aspects of ethanol. Interestingly, an unexpected ~20% increase in hypermobility was observed after three cycles of binge-like ethanol drinking. Although the FST is most frequently used to model depression-like behavior, emerging evidence suggests that increased hypermobility during the FST could be indicative of an inability to cope in a stressful situation, suggesting that repeated ethanol exposure in the present experiment transiently enhances stress reactivity.

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Keywords

binge drinking; forced swim test; depression-like behavior; anhedonia; taste reactivity; stress reactivity

1. INTRODUCTION

Under the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), alcohol use disorders (AUDs) are defined as “a problematic pattern of alcohol use leading to clinically significant impairment or distress... (American Psychiatric et al., 2013)”. Given that distress is a defining characteristic of AUDs, it is no surprise that AUDs rarely occur in isolation but are often accompanied by other disorders characterized by psychological distress. In fact, AUDs have been reported to have a relatively high comorbidity with both anxiety and major depressive disorders (Odlag et al., 2016; Swendsen et al., 1998). It has even been argued that frequent alcohol (ethanol) use may precipitate these states of psychological distress as it is believed that repeated stressors- such as binge drinking episodes- may cause neuroadaptive changes that leaves the individual vulnerable to depression (Beauchaine et al., 2011; Brady and Sinha, 2005; McEwen, 2000).

In line with this reasoning, a well-established model of ethanol dependence, chronic intermittent ethanol (CIE) vapor exposure, leads to plastic changes in key neuropeptide systems such as corticotropin releasing factor (CRF) and neuropeptide Y (NPY; (Eisenhardt et al., 2015; Sommer et al., 2008; Walker et al., 2010). Not only are these alterations believed to be indicative of a transition to a dependence-like state (Koob, 2003), these neuropeptide systems have also been implicated in contributing to both stress/anxiety and depression (Amstadter et al., 2010; Binder and Nemeroff, 2010; Mickey et al., 2011; Soleimani et al., 2014). Accordingly, animals exposed to ethanol vapor subsequently display decreased latency to immobility and increased immobility time during the forced swim test (FST) (Ehlers et al., 2011; Walker et al., 2010) as well as reductions in the time spent in the open arms during an elevated zero maze procedure (Kliethermes et al., 2004; Valdez et al., 2002). Moreover, these animals exhibit elevated levels of ethanol intake following ethanol vapor exposure (Valdez et al., 2002).

Using “drinking-in-the-dark” (DID) procedures to model binge-like ethanol drinking, we and others have found that repeated episodes of binge-like ethanol drinking lead to similar changes in CRF and NPY systems (Lowery-Gionta et al., 2012; McClintick et al., 2015; Sparrow et al., 2012; Sparta et al., 2013). Given that alterations in these peptide systems following DID procedures are analogous to those stemming from CIE, it stands to reason that the DID model of binge-like ethanol drinking would produce animals that similarly exhibit anxiety-like or depression-like phenotypes, as well elevated levels of voluntary ethanol intake. Published data from our lab provided partial support for this hypothesis as repeated episodes of binge-like ethanol drinking using DID procedures augmented subsequent ethanol consumption, a phenotype also characteristic of models of ethanol dependence, but these animals did not display anxiety-like phenotypes across a variety of paradigms (Cox et al., 2013); however, depression-like behaviors were not assessed. Thus,

the purpose of the present study was to investigate whether repeated episodes of ethanol DID promote a depression-like phenotype. Additionally, we employed procedures to assess anhedonia in mice with a history of binge-like ethanol drinking as anhedonia has been reported in individuals with AUDs (Hatzigiakoumis et al., 2011; Martinotti et al., 2008).

2. METHODS

2.1 Animals

Male C57BL/6J mice (n = 40), approximately 6–8 weeks old, were obtained from Jackson Laboratories (Bar Harbor, ME). Prior to experimentation, mice were given two weeks to acclimate to being individually housed in a vivarium with a reversed 12:12 hour light:dark cycle at approximately 22°C. The reversed light cycle allowed us to perform all experiments during the animals' dark cycle. Mice were allowed *ad libitum* access to T.2920X diet (Harlan[®] Laboratories, Inc.; Indianapolis, IN) and water unless otherwise indicated in experimental procedures (Marshall et al., 2015). All experimental procedures were approved by the UNC IACUC and complied with the NIH Guide for Care and Use of Laboratory Animals (NRC, 2011).

2.2 Drinking-in-the-Dark (DID) Paradigm

Binge-like drinking was modeled using the standard, four-day DID paradigm (Thiele and Navarro, 2014). Briefly, homecage water bottles were removed three hours into the dark cycle and replaced with sipper-tube bottles containing 20% (v/v) ethanol solution made from 95% ethanol (Decon Labs; King of Prussia, PA) and tap water. For days 1–3, ethanol bottles remained on the cages for two hours; however, on the fourth day, mice had four hours of access to the ethanol solution. Together, these four days constitute a single cycle of DID. Mice were randomly assigned to groups that experienced either 1, 3, or 6 cycles (n=10/group) of the 4-day binge with a three day rest period between cycles. The volume consumed was converted to grams of ethanol and divided by the body weight of the mouse to determine the ethanol dose (g/kg). A fourth group of control animals (n=10) had homecage water bottles replaced with sipper-tube bottles of tap-water for 6 cycles. Blood samples were not collected in an attempt to avoid any confounding effects of the stress of tail nicks and restraint on subsequent behavioral tasks (Harris et al., 2004; Tuli et al., 1995). A schematic outlining the procedure employed is presented in Figure 1

2.3 Forced-Swim Test (FST)

Approximately 24 hours following the last binge-like drinking session, mice were subjected to a forced-swim test (FST), which is a common method employed to assess depressive-like phenotypes in mice (Bogdanova et al., 2013; Porsolt et al., 1978). Procedures for the FST were similar to previous reports (Lowery et al., 2008; McCall et al., 2013). Briefly, mice were individually placed into a four-liter beaker (25 cm in height, 17.5 cm in diameter) filled with approximately 2.3 liters of room temperature water (21°C) for five minutes approximately three hours into the dark cycle to simulate the chronology of drinking conditions in the DID procedures. Mouse behavior was video-recorded and subsequently analyzed with EthoVision XT 7 (Noldus Information Technology, Inc.; Leesburg, VA). Using this EthoVision software, movement was characterized in three ways: immobility,

mobility, and hyper-mobility. This procedure has been described previously (Hedou et al., 2001; Juszczak et al., 2008; Juszczak et al., 2006). Briefly, the software identifies the pixels within the frame belonging to the animal and tracks these pixels frame-by-frame. Movement is assessed by comparing the location of these pixels from one frame to the next. The percentage of pixels that have moved locations is used to determine mobility state (i.e. immobile, mobile, or hyper-mobile) based on predetermined thresholds. In short, the percentage of pixels that have relocated directly reflects the amount of movement such that the more pixels that have changed location, the more the animal is moving. Immobility was defined as movement below 11.5%; whereas hyper-mobility was defined as movement above 18% based on the parameters suggested by EthoVision and previous reports (Bigio et al., 2016; Juszczak et al., 2008).

2.4 Anhedonia Test

One week following the last binge-like drinking session, anhedonia-like behavior was determined using a two-bottle sweet preference test. Three hours into the dark cycle, mice were given six hour access to two drinking bottles containing either a 1% (w/v) sucrose solution or a second bottle containing tap water (Bachmanov et al., 2001). A sucrose preference ratio (sucrose consumed/sucrose + water consumed) was calculated for each animal. Reduced sucrose preference is considered to be reflective of an anhedonia-like state (Willner et al., 1987).

2.5 Two-Bottle Voluntary Consumption and Preference Tests

Twenty-four hours after the anhedonia test, mice were given continuous access to two bottles. One bottle contained water while the other had either a sweet (sucrose) or bitter (quinine) solution. Daily consumption (g/kg) was measured for each concentration of sucrose (1, 3, or 6%, w/v) over a four-day period, in an ascending order. On day one of the 1% sucrose test, the bottles were disturbed affecting the final volume measure; therefore, the 1% sucrose test was only measured over a three-day period with the first day being excluded from the analysis. After a two-day washout period, mice were then similarly tested with quinine solution (0.03mM or 0.1mM. In addition to consumption, preference for either sucrose or quinine was determined using the same formula used in the anhedonia test.

2.6 Statistical Analysis

Prism Version 7.02 (GraphPad Software, Inc. La Jolla, Ca) was used to analyze and graph all data reported herein. One-way analyses of variance (ANOVAs) were used to determine the effect of ethanol experience (control, 1 cycle DID, three cycle DID, and six cycle DID) on binge-like ethanol consumption and activity during the FST. Repeated measures ANOVAs were employed for all other tests. Specifically, concentration of the tastant (1%, 3%, and 6% sucrose, or 0.03mM and 0.1mM quinine) served as the within-subject variable during the tests of voluntary sucrose and quinine consumption, respectively, while test day (days 1 and 2 versus days 3 and 4) was the within-subject variable for the quinine preference test and time (hour 1, 2, 3, 4, 5, and 6) was the within-subject variable for the anhedonia test. In all of these analyses, ethanol experience (control, 1 cycle DID, three cycle DID, and six cycle DID) was the between-subject variable. *Post hoc* Bonferroni tests with a correction for multiple comparisons were conducted if a significant interaction or main effect of the

number of DID cycles was observed. All data are reported as the mean \pm standard error of the mean and considered significant if $p < 0.05$, two-tailed.

3. RESULTS

3.1 DID Procedures Produce Similar Levels of Ethanol Consumption

The average ethanol consumption of all animals was 4.49 ± 0.2 g/kg. A one-way ANOVA comparing each of the DID cycle groups revealed no change in ethanol consumption on the final test day of ethanol access ($[F_{(2,27)} = 1.42, p = 0.26]$; Table 1). The similar levels of ethanol consumption between the groups experiencing different numbers of DID cycles is consistent with previous findings from our lab (Cox et al., 2013). However, the mice in the current study drank relatively less ethanol than mice from previous work (Cox et al., 2013; Olney et al., 2015; Sprow et al., 2015) but were similar to other DID reports with BECs above 80mg/dL (Lee et al., 2015; Marshall et al., 2016a; Marshall et al., 2016b). Differences in consumption between studies were most likely due to changes in rodent chow diet used at UNC (Marshall et al., 2015).

3.2 DID Cycle Experience alters High-Mobility in Forced-Swim Test

One-way ANOVAs indicated the number of DID cycles animals experience had no effect on either mobility (Figure 2A; $[F_{(3,36)} = 1.05, p = 0.38]$) or immobility (Figure 2B; $[F_{(3,36)} = 1.75, p = 0.18]$); however, in relation to high-mobility, a one-way ANOVA indicated that there was a significant effect of treatment (Figure 2C; $[F_{(3,36)} = 2.93, p = 0.047]$). *Post hoc* Bonferroni tests indicate that after 3 cycles of DID mice exhibited greater hyper-mobility than mice of the control group but were no different from either of the other ethanol treatment groups.

3.3 Ethanol Experience does not alter anhedonia state

Over the course of six hours, each group had an average sucrose preference of at least 75%, suggesting that they continued to find the 1% sucrose solution rewarding following a history of DID. A two-way (time x ethanol experience) repeated measures ANOVA revealed that sucrose preference changed over the course of the six hour test period (Figure 3A; [main effect of time: $F_{(5,180)} = 3.360, p = 0.006]$). However, further probing of this effect revealed that sucrose preference at any given time point did not reach statistical significance from any other time point after Bonferroni's correction of the alpha level. Furthermore, our analysis revealed marginal, albeit nonsignificant, effects of ethanol experience [main effect of ethanol experience: $F_{(3,36)} = 2.493, p = 0.076]$ and an interaction effect [time \times ethanol experience: $F_{(15,180)} = 1.635, p = 0.068]$.

3.4 Ethanol Experience Alters Tastant Consumption and Preference

In a two-bottle choice continuous access paradigm, mice with varied experiences with ethanol had distinct reactions to sweet and bitter solutions. A two-way repeated measures ANOVA of sucrose consumption showed a main effect of sucrose concentration [Figure 3B; $F_{(2,72)} = 383.640, p < 0.0001]$. Further probing of this effect revealed that increasing the concentration of sucrose resulted in increased consumption (1% vs 3%: 1% vs 6%; and 3% vs 6% $p < 0.0001$). There was a marginal, yet nonsignificant, effect of DID experience [$F_{(3,36)}$]

= 2.420, $p = 0.082$]. No significant interaction effect of DID experience and sucrose concentration was observed [$F_{(6,72)} = 1.522$, $p = 0.183$]. A two-way repeated measures ANOVA analyzing sucrose preference revealed a main effect of sucrose concentration [$F_{(2,72)} = 25.964$, $p < 0.0001$]. Further probing of this effect indicated that the 6% sucrose solution was significantly more preferred than any other concentration of sucrose (Figure 3C; 1% vs 6%; and 3% vs 6% $p < 0.0001$). However, no differences between the ethanol experience of groups [$F_{(3,36)} = 1.333$, $p = 0.279$] or an interaction [$F_{(6,72)} = 1.265$, $p = 0.284$] were observed.

In regard to consumption of the bitter, quinine solution, a two-way repeated measures ANOVA revealed a significant main effect of ethanol experience (Figure 3A; [$F_{(3,36)} = 3.00$, $p = 0.043$]), a significant main effect of quinine concentration [$F_{(1,36)} = 38.04$, $p < 0.001$], as well as a significant interaction effect between the two variables [$F_{(3,36)} = 3.19$, $p = 0.035$]. *Post hoc* Bonferroni tests revealed that the six-cycle DID group consumed significantly more 0.1mM quinine than both the control group ($p = 0.016$) and one cycle DID group ($p = 0.002$) while no other comparison was significant. Examining the preference for quinine compared with water, a two-way repeated measures ANOVA, indicated a main effect of ethanol experience [$F_{(3,36)} = 3.87$, $p = 0.017$] but not quinine concentration [$F_{(1,36)} = 2.14$, $p = 0.15$] and an interaction between the two variables ($F_{(3,36)} = 8.92$, $p = 0.020$); Figure 3B. Further probing of the interaction using *post hoc* Bonferroni tests revealed that at the 0.1mM concentration, the six-cycle DID group preferred quinine more than both the water-control animals ($p = 0.002$) and the one cycle DID animals ($p = 0.0008$); however no effects of ethanol experience were observed at the 0.03mM concentration.

4. DISCUSSION

This study sought to elucidate the impact of binge-like alcohol consumption on depression and anhedonic-like phenotypes under non-dependent conditions. Findings from the present study suggest that repeated cycles of binge-like ethanol drinking using DID procedures did not induce a depression-like phenotype in the FST as measured by immobility nor did it alter anhedonia as measured by voluntary sucrose consumption. Ethanol consumption in the DID paradigm did not lead to a state of anhedonia as neither sucrose preference nor voluntary sucrose consumption was significantly altered as a function of binge-like ethanol drinking, similar to a previous report (Lee et al., 2015). However, it should be noted that anhedonia may not be a core feature of depression (Treadway and Zald, 2011). Indeed, when measured objectively, patients with depression display intact hedonic reactions to sweet tastes (Berlin et al., 1998; Dichter et al., 2010). Notably, these results suggest that our previous observation of increased voluntary ethanol intake stemming from prior binge-like ethanol drinking (Cox et al., 2013) is not likely related to altered sensitivity to salient reinforcers or caloric need. Despite the lack of group differences in sucrose intake, we did observe a trend for elevated consumption of the higher concentration of quinine (0.1 mM) which was further supported by an increase in preference among animals that experienced six cycles of DID. Although this may reflect a reduction in sensitivity to bitter tastants, these differences in quinine responding may arise from a learned association formed by these animals to the taste ethanol (Bachmanov et al., 1996). Indeed, using taste reactivity to measure gustatory perception, Kiefer and colleagues demonstrated that rodents perceive the taste of ethanol as

possessing a bitter component (Kiefer et al., 1990). In this sense, repeated binge-like ethanol drinking may have promoted a learned association between the perceived bitter taste of ethanol and its reinforcing post-ingestive effects. When introduced to quinine, these animals may have perceived the bitter tasting solution as ethanol.

Although the focus of these studies was on depressive-like symptoms induced by binge-like consumption, it is important to recognize that we did observe a peculiar increase in high-mobility behavior among mice that experienced three cycles of ethanol DID. It is difficult to truly interpret this finding given that the increased hypermobility observed herein was transient and not seen in animals that experienced six cycles of ethanol. Although the FST is a widely used paradigm for depressive-like behaviors, the validity and translatability of this test to human behaviors continues to be of interest. A review by Commons and colleagues indicates that many of the behaviors previously attributed to depression may actually reflect coping mechanisms in response to the stress during the FST (Commons et al., 2017). Although rather unexpected, the increase in hypermobility observed in these studies may actually reflect altered stress-reactivity in these mice. More specifically, it could indicate that a history of binge-like ethanol drinking promotes maladaptive stress responding of the mice, which caused them to display hyper-reactive behavior (i.e. hypermobility) in response to the stress associated with the FST (de Kloet and Molendijk, 2016). This interpretation is supported by the fact that increases in CRF immunoreactivity within the central nucleus of the amygdala (CeA), an area known to be integrally involved in both alcohol consumption and stress/anxiety (Gilpin et al., 2015; Lowery et al., 2010; Rinker et al., 2016), were evident following three cycles of ethanol DID (Lowery-Gionta et al., 2012). Moreover, alterations in the NPY system within the CeA emerge after a single cycle of DID and peak after three cycles (Sparrow et al., 2012). Given that our previous study did not suggest altered stress-related phenotypes following DID using the elevated-plus maze or open field locomotor chambers (Cox et al., 2013), we believe the hypermobility observed herein may suggest a maladaptive response to a stressor or panic-like phenotype (Becker et al., 2011; Breese et al., 2005; Lowery et al., 2008). Moreover, a recent report by Somkuwar and colleagues demonstrate through both biological and behavioral indicators that irritability responses are heightened after withdrawal, suggesting that ethanol dependence may sensitize stress-reactivity (Somkuwar et al., 2017). Interestingly, the effects on irritability and anxiety observed appear to be dependent upon the duration of abstinence (Somkuwar et al., 2017; Zhao et al., 2007). Whether the stress associated with FST compounds stress reactivity similar to the stress of withdrawal remains to be determined. To truly understand this anomalous result, future studies will have to compare stress reactivity within the context of non-dependent ethanol consumption during intoxication as well as during different durations of abstinence using tests such as acute startle response or tail suspension task.

Together with the previous findings from our lab (Cox et al., 2013), the present study indicates that a history of binge-like ethanol drinking resulting from DID procedures is not significantly associated with anhedonia, anxiety-, or depression-like behavior. Intriguingly, these findings contrast with a recent study that used similar methods yet found drastically different results. Using modified DID procedures, Lee and colleagues reported that a history of binge-like ethanol drinking produced increases in anxiety-like behavior as measured by a variety of assays, including the elevated plus maze, as well as signs of depression-like

phenotypes via the FST (Lee et al., 2015). Notably, these authors did not measure hypermobility during FST. Whether or not the DID paradigm employed by Lee and colleagues similarly induced a hyperactive state during the FST remains unknown. Although the procedures of the behavioral assessments used in our studies versus those previously used were slightly different (Lee et al., 2015), the contrasting behavioral outcomes are most likely a result of the different DID procedures used by each research group. Indeed, we employed the standard, 4-day DID model that consists 2-h access to ethanol during the first three days and 4-h access on the fourth day followed by three days of rest between cycles in our studies. On the other hand, Lee and colleagues used a modified, 5-day procedure in which animals had 2-h of access to ethanol across the five days with only two days of rest between cycles (Lee et al., 2015). Moreover, our 5-minute FST was done 24 hours after the last ethanol cycle, but Lee and colleagues first used a 15-minute FST and report that the anxiety measures increased with prolonged abstinence (22 days after ethanol exposure) (Lee et al., 2015). It is possible that by using a longer FST or by putting animals through prolonged forced ethanol abstinence, Lee's studies may have observed heightened depression and anxiety. It is also possible that a depression-like phenotype may have emerged had we performed the FST temporally more proximal to the removal of ethanol.

It is important to continue to differentiate the neurobiological maladaptations and behavioral consequences induced by alcohol dependence compared with binge drinking to understand the transition between alcohol misuse and dependence. Reports continue to demonstrate that neurobiological changes that underlie the transition to a dependent-like state may occur as a result of repeated binge exposure. For example, CIE, a model of alcohol dependence, and the DID binge-model have been reported to lead to similar changes in key neuromodulatory systems associated with dependence and psychological distress (Eisenhardt et al., 2015; Lowery-Gionta et al., 2012; McClintick et al., 2015; Sommer et al., 2008; Sparrow et al., 2012; Sparta et al., 2013; Walker et al., 2010). However, the behavioral outcomes resulting from these two paradigms are not identical. Although each of these models promotes increases in subsequent ethanol consumption (Cox et al., 2013; Gilpin et al., 2011), animals that experience CIE display stress-related phenotypes (Ehlers et al., 2011; Kliethermes et al., 2004; Somkuwar et al., 2017; Valdez et al., 2002) while those that experience DID do not consistently show stress-related phenotypes (Cox et al., 2013; Lee et al., 2015). Findings from the present study extend upon these findings by demonstrating that, unlike CIE procedures (Walker et al., 2010), repeated cycles of binge-like ethanol drinking using DID procedures did not support a depression-like phenotype in the FST. Importantly, both models promote subsequent increases of voluntary ethanol consumption. When taken together, these findings support DID as an ideal model for studying the early stages of neuroplasticity that emerge with repeated bouts of binge-like ethanol intake and that continue to develop over the transition to dependence.

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Highlights

- Non-dependent binge-like consumption did not alter depressive-like behaviors
- Binge drinking resulted in increased quinine consumption
- Binge drinking caused a transient increased hyper-reactivity response in the forced-swim test

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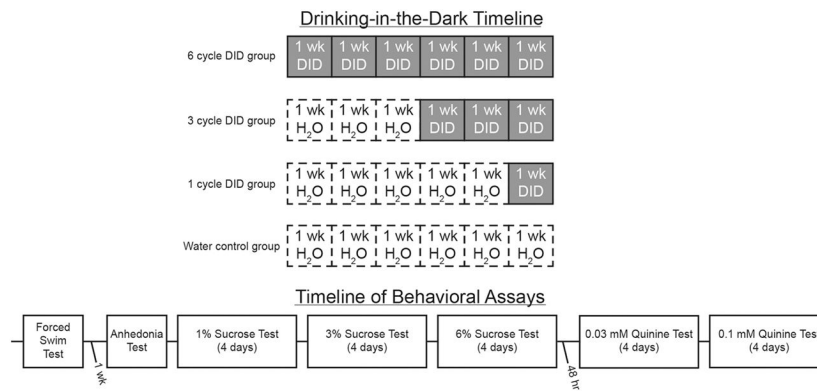


Figure 1. Timeline of experimental procedures

Top: mice were randomly assigned to one of four groups that experienced six, three, or one week(s) of ethanol DID or a water control group that did not experience ethanol DID (n=10/group). Groups were staggered such that all animals completed DID on the same day.

Bottom: 24 hours after the final DID session, mice began the battery of behavioral assays to assess depression-like phenotypes. Time between each assay was 24 hours unless otherwise stated. Abbreviations: DID = drinking-in-the-dark; wk = week.

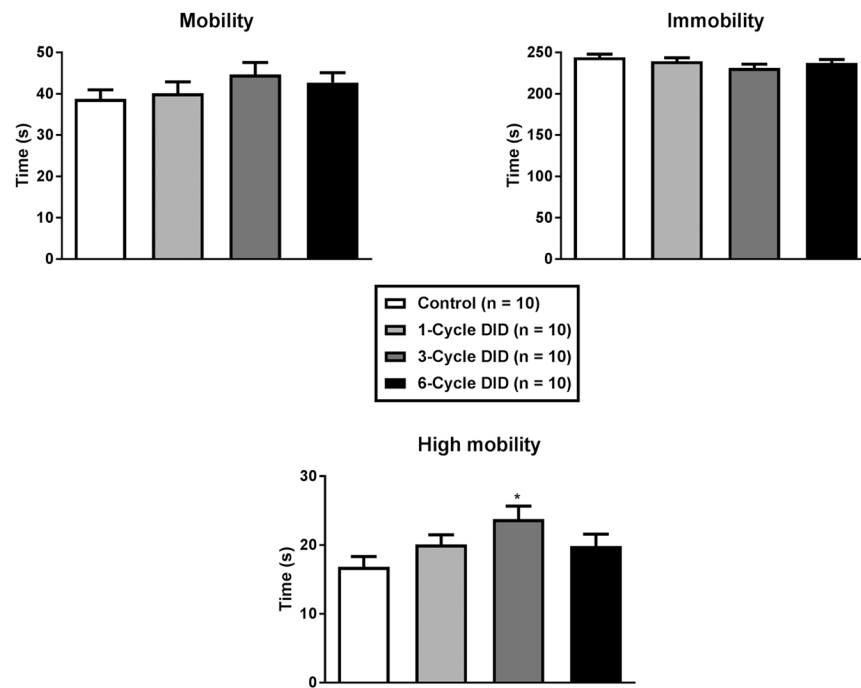


Figure 2. Average mobility during the 5-minute forced-swim test

Relative to water drinking control animals, there were no significant effects of binge-like drinking history (1 to 6 binge cycles) on mobility (**A**) or immobility (**B**) measures.

Interestingly, relative to controls, mice that experience 3 DID cycles showed elevated levels of high mobility behavior (**C**; defined as 18% greater activity than the average of mobility behavior). All values are means \pm SEM, and * $p < 0.05$ relative to control.

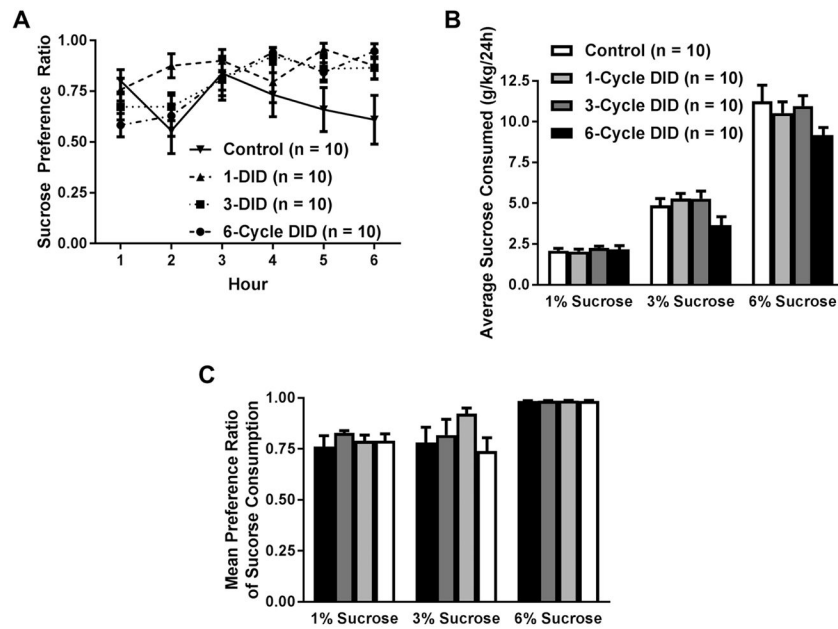


Figure 3. Average hourly sucrose preference ratio (sucrose intake/total fluid intake) and daily sucrose consumption in groups of mice that drank water (control) or had a prior history of 1, 3, or 6 cycles of DID

A prior history of binge-like ethanol drinking did not alter sucrose preference during the anhedonia test (A). No effect of ethanol experience was observed on sucrose consumption, but animals with access to 6% sucrose consumed more sucrose than the other concentrations (B). Moreover, all animals preferred the sweet taste of high concentration of sucrose (6%) more than either of the other two concentrations regardless of DID history (C). All values are means \pm SEM.

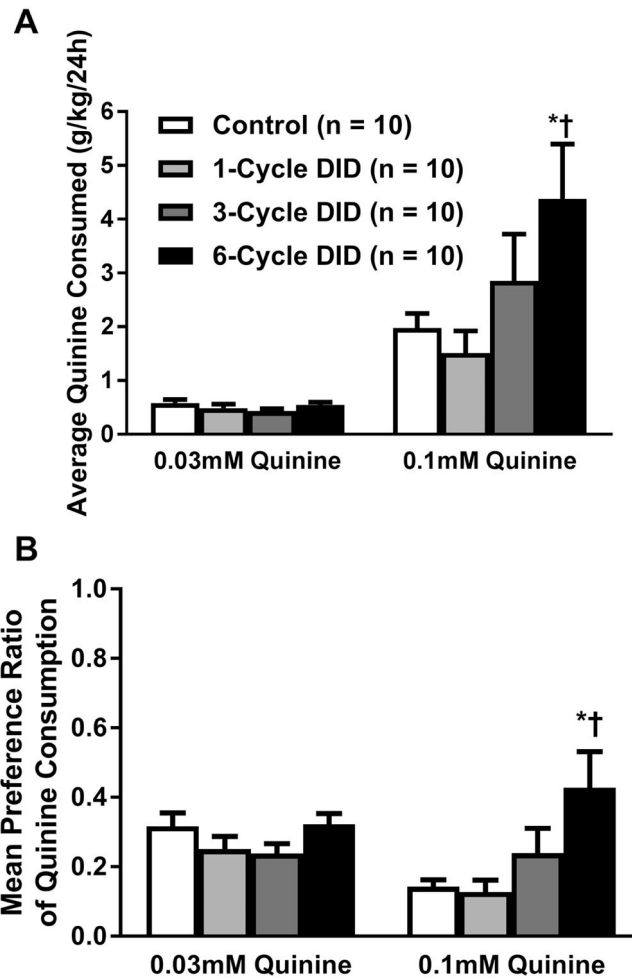


Figure 4. Average daily consumption (g/kg/24h) of each concentration of quinine solution, or average preference ratio of the 0.1 mM quinine solution, by control mice or mice that had a prior history of 1, 3, or 6 cycles of DID

Animals in the 6-cycle DID group displayed significantly elevated voluntary consumption (A) and preference (B) of quinine at the 0.1 mM concentration relative to both the control and 1-cycle DID groups. All values are mean \pm SEM; * signifies that $p < 0.05$ relative to the water-drinking control group; † denotes that $p < 0.05$ relative to 1-cycle DID group.

Table 1
Binge-like ethanol consumption (g/kg/4h) on the last day of ethanol access in mice that experienced 1, 3, or 6 cycles of DID

No significant differences in ethanol consumption were observed as a function of DID history. All values are means \pm SEM.

Ethanol Consumption (g/kg/4h)	
1-Cycle Ethanol	4.05 \pm 0.44
3-Cycle Ethanol	4.62 \pm 0.32
6-Cycle Ethanol	4.82 \pm 0.21

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