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Binge Alcohol Drinking Elicits a Persistent Negative Affective State in Mice

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by

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ABSTRACT

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In alcohol-dependent humans, cessation of alcohol consumption produces a dysphoric, anxiogenic state that can persist into protracted withdrawal. This aversive withdrawal-induced affective state is theorized to contribute to the negative reinforcing properties of alcohol that maintains and/or precipitates relapse in those suffering with alcoholism. Despite this and evidence that alcohol withdrawal increases biochemical indices of stress, there are relatively few published studies examining the impact of cessation from binge drinking on behavioral measures of negative affect. To this end, 60 C57BL/6 mice were allowed to consume alcohol under modified Drinking-in-the-Dark (DID) procedures, which consisted of access to a sipper tube containing either 20% alcohol or water for 2hrs/day, 5 days/week, for 6 consecutive weeks. Half of the mice from each drinking group were subjected to a 2-day behavioral test battery consisting of conventional assays of anxiety and depression either 24hrs (n=30) or 21 days (n=30) into withdrawal. A combined analysis revealed higher indices of anxiety assessed in the light/dark shuttle box, novel object encounter, elevated plus maze, and marble burying tests in alcohol-drinking (AD) compared to water-drinking (WD) controls, independent of withdrawal duration. AD mice also showed a shorter latency to float on the first day of the forced swim test, indicating a higher prevalence of behavioral despair; however, these mice also showed an increase in swimming behavior during the retest, possibly due to elevated reactivity to stressor re-exposure. The

present results indicate that increases in anxiety and depression emerge very early after cessation of drinking and persist into protracted withdrawal, suggesting that a history of heavy binge drinking produces enduring neuroadaptations within brain circuits mediating emotional arousal.

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Introduction

Binge drinking is the most common pattern of excessive alcohol consumption, with over 38 million Americans admitting to binge drinking an average of four times per month (National Center for Chronic Disease Prevention and Health Promotion, 2012). Binge drinking is defined as a pattern of alcohol intake sufficient to produce a blood alcohol concentration (BAC) of ≥80mg% in a 2hr period (National Institute on Alcohol Abuse and Alcoholism, 2004). Frequent binge drinking can result in physical dependence, leading to symptoms of withdrawal during periods of abstinence. Withdrawal symptoms often include insomnia, confusion, anxiety, depression, irritability, tachycardia, and in severe cases, delirium tremens and seizures (Bayard, McIntyre, Hill, & Woodside, 2004; Hall & Zador, 1997). Characterizing the neurobiological impact of binge drinking is crucial for the development of relevant pharmacotherapies for the treatment of this form of alcohol-use disorder (AUD).

Elevated anxiety and depression during alcohol withdrawal are frequently reported in the human population (Driessen et al., 2001; Hall & Zador, 1997). This aversive state is a compelling source of negative reinforcement that fuels compulsive drug seeking behavior and relapse (Koob, 2009). Alcohol-induced changes to the brain in regions collectively referred to as the extended amygdala have been implicated in the emotional dysregulation that can occur during alcohol abstinence. The extended amygdala is a basal forebrain macrosystem that acts as a subcortical relay station between the brainstem, thalamus, and cortical areas (Alheid, 2003; Alheid & Heimer, 1988). The extended amygdala includes the bed nucleus of the stria terminalis, which mediates fear and anxiety, the shell of the nucleus accumbens, which is involved in pleasure and reinforcement, and the central amygdala, which plays a role in depression (Alheid, 2003). As such, the extended amygdala is integrally

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involved in emotionality and dysregulation of the extended amygdala has also been implicated in drug reward and reinforcement (Koob, 1999; Koob & Le Moal, 2001).

Relatively few studies have looked at behavioral assays of anxiety and depression during withdrawal in animal models of alcohol abuse. In general, previous studies examining emotional dysregulation during alcohol withdrawal have typically relied on non-contingent alcohol delivery paradigms such as vapor inhalation, liquid diet, injection, or gavage (Kliethermes, 2005). While these methods are effective at producing physical dependence and robust effects upon behavioral indices of negative affect, such models arguably lack face validity and often rely on higher doses than animals would consume voluntarily (Becker, 2000; Egli, 2005; Freund, 1975; Hitzemann, 2000; Kirchhoff & Chester, 2013; McMillen, 1997; Spanagel, 2000). Even fewer studies have looked at emotional dysregulation during withdrawal from binge drinking specifically, despite a preponderance of evidence that binge drinking models are capable of producing withdrawal symptoms during periods of abstinence (Crabbe, Harris, & Koob, 2011).

Although limited, there exists some evidence from animal models to support a dysregulation of extended amygdala function during withdrawal from binge drinking. For instance, studies have shown increased indices of glutamate transmission within extended amygdala structures at 24 hrs following a month-long history of binge drinking (Cozzoli et al., 2012; Cozzoli et al., 2014). Other studies have found that binge drinking produces region-specific changes in gene expression related to synaptic transmission and neuronal plasticity within the NAc shell and CeA (Freeman et al., 2013; McBride et al., 2010). Thus, binge alcohol drinking may induce changes to this extended amygdala system similar to those reported in models of alcohol dependence and these neuroadaptations are a likely source of affective dysregulation during withdrawal also from binge drinking.

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In the current study, we sought to characterize the effects of binge drinking on anxiety and depression in adult mice using a modified version of the well-established "Drinking in the Dark" (DID) binge drinking model (Cozzoli et al., 2012; Crabbe et al., 2009; Moore & Boehm, 2009; Rhodes, Best, Belknap, Finn, & Crabbe, 2005). Several conventional behavioral assays of anxiety and depression were administered during short-term and long-term withdrawal in order to determine the persistence of these effects during prolonged abstinence.

Methods

Subjects

This study used 60 adult C57BL/6J male mice, 8 weeks of age at onset of drinking and weighing 25-30g (Jackson Laboratories, Sacramento, CA). Animals were divided into an alcohol-drinking group (n=30; hereafter referred to as AD mice) and a water-drinking group (n=30; hereafter referred to as WD mice). Subjects were individually housed in standard, Plexiglas cages, under a 12-hour-reverse light/dark cycle (lights off at 10am), in a temperature-controlled vivarium (23°C). Food and water were available *ad libitum.* All experiments were conducted in compliance with the National Institutes of Health *Guide for Care and Use of Laboratory Animals* (NIH Publication No. 80–23, revised 1996) and approved by the IACUC of the University of California, Santa Barbara.

Drinking-in-the Dark (DID) Procedures

To elicit consistently high alcohol consumption, we employed a modified version of the DID model, which has been shown to produce alcohol intakes between 3.5-5.0 g/kg alcohol in a 2-hour period and yield blood alcohol concentrations (BACs) in excess of 80mg% (Cozzoli et al., 2012; Crabbe et al., 2009; Moore & Boehm, 2009; Rhodes et al., 2005). Three

hours after lights out, home cage water bottles were replaced with sipper tubes containing a 20% (v/v) unsweetened alcohol solution in filtered tap water and allowed to drink for 2 hours, at which point the alcohol bottles were removed from the cages and the home cage water bottles were replaced. Control animals received a 50-ml sipper tube of filtered tap water *in lieu* of 20% alcohol. Mice were subjected to these drinking conditions 5 days per week over the course of 6 weeks (total drinking days = 30). Each day, the amount of alcohol consumed was calculated based on the weights of the bottles immediately before and after the drinking period. Unfortunately, at the time of study, technical difficulties with our Analox Analyzer precluded our ability to determine BACs in this group of animals. Thus, BACs were estimated from observed intakes and based on the results of published correlational analyses in B6 mice (Rhodes et al., 2005) as conducted previously (Cozzoli et al., 2009).

Behavioral testing

We administered test batteries to assay for alcohol withdrawal-induced changes in behavior in both the short-term (24-hr following last alcohol presentation) and long-term (21 days following last alcohol presentation) (n=30; 15 AD + 15 WD for each withdrawal time-point). At both time-points, testing began with an overnight test for sucrose preference and the remaining tests were conducted across the following 2 days. On the first test day after sucrose preference testing, mice were assayed in a light/dark shuttle box, novel object encounter, and a 15-min swim test. On the second test day, mice were tested on the elevated plus maze, marble burying, and a swim retest. Animals were sacrificed and brain tissue was harvested immediately following the swim retest on day 2. All tests were conducted under standard ambient lighting and the details of the procedures employed for each of these paradigms are provided below.

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Sucrose preference. Anhedonia, an absence of pleasure from previously enjoyable activities, is a characteristic symptom of depression in humans**.** Low sucrose preference is a well-established index of anhedonia in animal models (Katz, 1982) and thus, we examined for the effects of early versus late withdrawal from binge drinking on sucrose preference in our mice. For this, animals were given overnight access to 2 identical sipper tubes, one containing 5% sucrose and the other containing tap water. The bottles were weighed prior to being placed on the home cage at 17:00h. Sixteen hours later (09:00 h the next day), the bottles were removed from the home cage and weighed to determine the total volume consumed, as well as the relative preference for sucrose.

Light/dark shuttle box. The light/dark shuttle box test was used to assess exploratory and anxiety-like behaviors (Crawley & Goodwin, 1980; Hascoet & Bourin, 2009). Animals were placed into a Plexiglas box measuring 46 cm long×24 cm high×22 cm wide containing 2 distinct environments for a 15-minute trial. Half of the box was white and uncovered, the other half black and covered, separated by a central divider with an opening. The number of light-side entries and total time spent in the light side of the shuttle box were recorded using Any-maze™ tracking software (Stoelting co, Wood Dale, IL). Increased reluctance to venture into the light, uncovered side is interpreted as an index of anxiety.

Novel object encounter. To test reactivity to a novel object as an index of neophobia-related anxiety (Misslin & Ropartz, 1981), animals were placed in an activity box measuring 46 cm long×42 cm wide×40 cm high with a novel object in the center of the box- in this case, a colorful candlestick holder measuring approximately 6cm in diameter×12cm high. The

animals' interaction with the novel object was observed during a 2-minute trial. The number of contacts, total time spent in contact with the novel object, and fecal count were recorded by a trained observer who was blind to the drinking condition of the animals.

Porsolt forced swim test. Floating behavior during the Porsolt forced swim test serves as an index of behavioral despair in laboratory animals and is a model of depression with high predictive validity for the clinical efficacy of anti-depressant drugs (Karl, Pabst, & von Horsten, 2003; Porsolt, Bertin, & Jalfre, 1977). On day 1 of behavioral testing, each animal was placed into a 26cm diameter pool of room-temperature water deep enough so animals were unable to touch the bottom and behavior was monitored every 30 seconds by a trained observer for 15 min. On day 2 of behavioral testing (approximately 24 hrs following the first swim test), the animals were re-exposed to the pool for a 5-min retest session, in which behavior was monitored every 30 seconds. On both test days, the animal's behavior at each observation was classified as floating (all 4 limbs completely immobile), treading (minimal limb motion with no forward movement), or swimming (active paddling with forward movement). The latency to first float was also recorded using a stopwatch.

Elevated plus maze. The elevated plus maze is a well-established a measure of anxiety in laboratory animals (Walf & Frye, 2007). Animals were placed on the center intersection of a 4-arm radial plus maze with 2 white open arms and 2 black walled arms 24cm high. Each arm measured 123cm long × 5cm wide. Latency to first open-arm entry, number of open-arm entries, and total time spent in an open arm were monitored by a trained observer during a 4-minute trial. Changes in the amount of interaction with an open arm relative to an enclosed arm can be used to assess anxiety.

Marble burying. The marble burying test was used to measure anxiety-induced defensive burying (Misslin & Ropartz, 1981; Njunge & Handley, 1991). Twelve square marbles (2.5cm^2×1.25cm tall) were placed in the animals' home cage, 6 at each end. Latency to start burying the marbles was determined by an observer using a stopwatch and the total number of marbles buried following a 20-minute trial was recorded.

Statistical analyses

For each week of alcohol consumption, the average alcohol intake (expressed as g/kg body weight) was determined and data were analyzed using a within-subjects ANOVA with repeated measures on the Week factor (6 levels), with Fisher LSD post-hoc pairwise comparisons. Statistical analyses of all behavioral testing and cell counts were conducted using between-subjects two-way analysis of variances (ANOVAs), followed by post-hoc t-test comparisons when appropriate. α=0.05 for all tests. Calculations were performed using SPSS v.21 statistical software (IBM, 2012).

Results

Alcohol intake under Drinking-in-the-Dark procedures

The B6 mice in this study consumed on average 4.0±0.06 g/kg/2 h over the entire 6-week drinking period (Fig.1). Although blood alcohol levels were not assayed in this study, this level of intake is predicted to result in BACs ≥80 mg%, based on published results (Rhodes et al., 2005). As illustrated in Fig.1, the alcohol intake of the animals was more, rather than less, stable across the 6 weeks of drinking, although the repeated measures ANOVA across

all 6 weeks indicated some fluctuation [week effect: $F_{(5,145)}=15.48$, $p<0.001$]. Fisher LSD

post-hoc comparisons revealed lower intakes in week 1 and a transient spike in week 4

(pairwise p's<0.05). Despite these fluctuations, alcohol intake was persistently high

throughout the 6-week exposure phase. Importantly, there was no difference in the average

amount of alcohol consumed between animals assigned to be tested for behavior during short

versus long-term withdrawal [$t(28)=0.304$, $p>0.05$].

Sucrose preference

No group differences in sucrose preference or intake were observed following the

overnight sucrose preference test (2-way ANOVAs, p's>0.05).

Novel object

Mice with a history of binge drinking exhibited less exploration of a novel object during

withdrawal compared to alcohol-naïve mice (Fig.2a-b). AD mice made fewer contacts with

the object [Treatment effect: $F_{(1,48)}=12.74$, $p=0.001$] and spent less time interacting with

the object [Treatment effect: $F_{(1,44)}=6.31$, $p=0.016$] during the 2-minute trial. These main

treatment effects were independent of withdrawal duration (no withdrawal effect or

interaction, p's>0.05). There were no significant group differences in the latency to first

contact or in fecal count (2-way ANOVA, p's>0.05).

Elevated plus maze

During withdrawal from binge drinking, AD mice showed a significantly longer latency

to first open-arm entry compared to WD controls (Fig. 3)[Treatment effect: $F_{(1,46)}=7.60$,

$p=0.008$]. There were no group differences in the total number of open-arm entries, full entries, or total time spent in an open arm (2-way ANOVAs, $p's>0.05$).

Marble burying

AD mice exhibited an increase in marble burying compared to WD controls, independent of withdrawal duration (Fig. 4) [Treatment effect: $F_{(1,51)}=13.24$, $p=0.001$; no main withdrawal effect or interaction, $p's>0.05$]. There were no significant group differences in the latency to begin burying (2-way ANOVA, $p's>0.05$).

Porsolt forced swim test

AD mice had a shorter latency to first float during the 15-minute trial on test day 1 compared to WD mice (Fig. 5a) [Treatment effect: $F_{(1,44)}=10.38$, $p=0.002$]. There were no group differences in swimming, treading, or floating on test day 1 (2-way ANOVAs, $p's>0.05$). During the 5-minute re-exposure test on day 2, AD mice showed significantly more swimming (Fig. 5c) [Treatment effect: $F_{(1,45)}=16.32$, $p<0.001$] and less treading (Fig. 5c) [Treatment effect: $F_{(1,51)}=5.77$, $p=0.02$] than WD mice. Again, the treatment differences did not vary as a function of withdrawal duration (no Withdrawal effect or interaction, $p's>0.05$). There were no group differences in latency to first float or total number of floats on test day 2 (2-way ANOVAs, $p's>0.05$).

Interestingly, AD mice showed an increase in the overall proportion of swim observations between test day 1 and 2 compared to WD mice (Fig. 5d) [Treatment effect: $F_{(1,45)}=10.15$, $p=0.003$]. A one-sample t-test on each group confirmed that AD mice showed a significant increase in swimming behavior between test day 1 and 2 [$t_{(27)}=2.09$, $p=0.046$], while, in contrast, the WD mice showed a significant decrease [$t_{(20)}=2.92$,

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p=0.008]. The overall proportion of swim observations did not vary systemically with withdrawal duration (no withdrawal effect or interaction, *p*'s>0.05).

Light/dark shuttle box

Binge drinking influenced the number of entries into the light-side of a light/dark shuttle box, but the magnitude of this effect was dependent upon the withdrawal period (Fig. 6a) [Treatment effect: $F(1,44)=22.27$, $p<0.001$; Treatment X Withdrawal: $F(1,44)=5.10$, $p=0.029$]. Deconstruction of the significant interaction for light-side entries along the withdrawal factor indicated that the AD mice made fewer light-side entries than WD mice at both withdrawal time points [Short-term: $t(19)=3.742$, $p=0.001$; Long-term: $t(25)=2.496$, $p=0.023$], with a more prominent group difference during short-term withdrawal as is apparent in Fig. 6a. While there were no time-dependent differences within the AD mice [$t(22)=1.13$, $p>0.05$], WD mice showed a trend for a main duration effect [$t(22)=1.953$, p=0.064). This latter result was likely the source of the interaction effect revealed by the 2-way ANOVA. The cause of this trend is uncertain, but for the purposes of this study, it is most relevant that AD mice made fewer light-side entries at both time points and their behavior remained constant at both time points. Overall, AD mice also traveled a greater distance in the light side compared to WD controls (Fig. 6b) [Treatment effect: $F(1,43)=6.9$, $p=0.012$; interaction: $p>0.05$]. There were no group differences in the latency to first light-side entry or total time spent in the light side (2-way ANOVAs, *p's*>0.05).

Discussion

Many alcoholics report that anxiety reduction is a key motivator for drinking (Kushner, Abrams, & Borchardt, 2000). Therefore, increased anxiety is a negative reinforcer

that substantially increases the likelihood of relapse during abstinence from chronic alcohol abuse (Cloninger, 1987; Koob & Le Moal, 1997). Individuals who regularly engage in heavy episodic drinking such as binge drinking do not necessarily meet the diagnostic criteria for alcoholism (National Center for Chronic Disease Prevention and Health Promotion, 2012); however, frequent binge drinking is a significant risk factor for the development of alcoholism and can produce similar symptoms during withdrawal (Hasin, Stinson, Ogburn, & Grant, 2007; Knight et al., 2002; Substance Abuse and Mental Health Services Administration, 2011). Feelings of dysphoria during cessation from binge drinking promote re-engaging in the behavior in order to alleviate these symptoms. Perpetuation of this cycle likely contributes to the transition from binge drinking to alcoholism.

In the present study, we show that 6 weeks of voluntary binge drinking under modified DID procedures is sufficient to elevate indices of anxiety and depression during withdrawal in a murine model. These affective changes are present as early as 24-hrs into withdrawal and persist for at least 3 weeks following the last exposure to alcohol.

Elevated indices of anxiety during withdrawal from binge drinking

Binge drinking produces anxiogenic effects during withdrawal, as evidenced by treatment-dependent differences on the elevated plus maze, novel object encounter, marble burying task, and light/dark shuttle box test. The AD animals showed greater reluctance to interact with a novel object compared to WD animals, suggesting heightened levels of neophobia-induced anxiety. This was further supported by the increased burying behavior in AD mice compared to WD mice in the marble burying test. These results are consistent with a previous study showing increased neophobia during withdrawal from an alcohol liquid diet in rats (Knapp, Saiers, & Pohorecky, 1993). In that study, animals engaged in 4 days of

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alcohol consumption and showed intakes of 7-13g/kg. Heightened neophobia was observed 10-30 hrs into withdrawal. However, this effect had dissipated by 70 hrs. Despite high alcohol consumption, the brief duration of alcohol exposure this study was likely insufficient to elicit the enduring behavioral changes seen in the present study.

On the elevated plus maze, AD mice showed a significantly longer latency to first open-arm entry compared to WD controls, indicating increased anxiety in response to open and brightly lit areas. These results are consistent with many previous studies on the anxiogenic effects of alcohol withdrawal in both rats and mice (Doremus, Brunell, Varlinskaya, & Spear, 2003; Jung, Wallis, Gatch, & Lal, 2000; Onaivi, Todd, & Martin, 1989). A similar aversion was seen in the light/dark shuttle box test, with AD mice showing fewer light-side entries at both withdrawal time-points. Surprisingly, AD mice traveled a greater distance in the light side compared to WD mice, despite fewer entries. Since there were no group differences in the total time spent in the light side, these data suggest that AD mice exhibited a longer duration per entry and greater hyperactivity in this aversive environment, compared to WD mice.

A primary effect of acute alcohol is a potentiation of inhibitory GABA neurotransmission and a suppression of excitatory glutamate transmission. Repeated alcohol exposure is known to cause a compensatory down-regulation of GABA receptors and an increase in excitatory glutamatergic transmission via upregulation of both NMDA receptors (Hughes, 2009) and metabotropic glutamate receptors (Cozzoli et al., 2012; Cozzoli et al., 2014; Cozzoli et al., 2009). These changes produce a state of CNS hyperexcitability during withdrawal from chronic alcohol consumption. The increase in motor activity on the light/dark box could be a sign of psychomotor agitation due to this GABA-glutamate imbalance (Esel, 2006).

Locomotor hyperactivity during withdrawal, especially in response to a novel environment, has also been associated with altered HPA-axis function and an increased sensitivity to the dopamine-stimulating effects of glucocorticoids (Koob & Le Moal, 1997; Piazza & Le Moal, 1996). Psychomotor dysregulation is a common symptom in humans during alcohol cessation (Saitz & O'Malley, 1997) and can be a symptom of anxiety (Sadock, Sadock, Ruiz, & Kaplan, 2009) and certain depression subtypes (Sadock et al., 2009; Sobin & Sackeim, 1997). Thus, our data for both neophobia and motor hyperactivity are consistent with and extend extant data from humans and laboratory animals to a murine model of binge alcohol drinking, providing further predictive validity for this model of alcohol abuse.

Elevated indices of depression during withdrawal from binge drinking

This study found evidence of withdrawal-induced depressive symptoms, indicated by a shorter latency to float in the Porsolt forced swim test for mice with a history of binge drinking. Strangely, AD mice showed significantly more swimming and less treading than WD mice during the 5-minute re-exposure test on day 2. Further analysis of this effect also showed an increase in the overall proportion of swim observations between test day 1 and 2 in AD mice, while in contrast, the WD mice showed a significant decrease. Increased floating during the re-exposure test seems to be a more adaptive response in order to conserve energy, since the animals have prior experience and are familiar with futility of escape efforts. In AD mice, this maladaptive increase in swimming behavior might reflect heightened anxiety, provoking a panicked frenzy in response to a previously stressful situation (Quintino-dos-Santos et al., 2014). Increased reactivity to stressor re-exposure would be consistent with the data from our anxiety measures indicating a persistent increase in anxiety in AD animals.

We failed to detect AD-WD differences in the sucrose preference test – a test widely employed for assessing anhedonia in rodents (Katz, 1982). Initially, this result seemed surprising, given that AD mice exhibited signs of increased depression on the forced swim test. However, many studies have shown a positive correlation between alcohol consumption and increased preference for sweet solutions in laboratory animals (Gosnell & Krahn, 1992; Stewart, Russell, Lumeng, Li, & Murphy, 1994) and humans (Kampov-Polevoy, Garbutt, & Janowsky, 1997; Kranzler, Sandstrom, & Van Kirk, 2001). Some studies have even noted an increase in sucrose consumption during withdrawal from chronic alcohol exposure persisting at least 4 weeks into withdrawal (Rasmussen et al., 2000; Rasmussen, Mitton, Green, & Puchalski, 2001). Given this and the other behavioral results, it is argued that the failure to observe any change in the high sucrose preference of AD mice during alcohol withdrawal may reflect insensitivity of the test, rather than no effect of alcohol withdrawal on anhedonia.

Proposed mechanisms underpinning the persistent negative affective state produced by alcohol withdrawal

There is a preponderance of evidence showing a disruption of homeostatic balance within the HPA axis during alcohol withdrawal. Increased norepinephrine (NE) and corticotropin-releasing hormone (CRH) signaling throughout the extended amygdala due to hyperactivity of the stress response system has been linked to the negative affective states experienced during periods of alcohol abstinence (Rasmussen et al., 2001; Roberto, Gilpin, & Siggins, 2012; Smith & Aston-Jones, 2008; Stephens & Wand, 2012). In fact, many stress-related changes in behavior seen during alcohol withdrawal can be reversed through local administration of NE or CRH antagonists within the extended amygdala (Linnoila, Mefford, Nutt, & Adinoff, 1987; Rassnick, Heinrichs, Britton, & Koob, 1993). These HPA axis

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abnormalities have been shown to persist into protracted withdrawal and play a role in increased drug seeking and stress-induced relapse (Rasmussen et al., 2000). A recent report also found evidence of BDNF-mediated negative affect following 12 days of binge-like drinking in adolescent rats (Briones & Woods, 2013). Therefore, there is preliminary evidence to support binge drinking- induced dysregulation of the HPA axis related to emotional dysfunction.

Dysphoric states during withdrawal are also mediated by changes in neurotransmission throughout the extended amygdala. In addition to a glutamate-GABA imbalance, decreased dopaminergic and serotonergic transmission also occurs within the nucleus accumbens during alcohol withdrawal (Weiss et al., 1996). As these deficits in monoaminergic neurotransmission have been linked to decreased brain reward function (Markou & Koob, 1991), they may contribute to the depressive effects of withdrawal from binge alcohol drinking. While a prior study of the effects of a history of binge drinking upon extracellular amino acid and monoamine neurotransmitter levels failed to detect changes within the nucleus accumbens (Szumlinski et al., 2007) the alcohol experience of the mice in this prior study was moderate (6 bouts) and considerably less than that herein (30 bouts). Thus, it still remains to be determined whether or not the negative affective state produced by a chronic history of binge drinking is associated with perturbations in amino acid and monoamine neurotransmitter systems within the extended amygdala.

It is important to note that alcohol withdrawal studies are typically conducted in animal models of chronic alcohol dependence. In the human population, binge drinking is the most prevalent form of alcohol abuse (National Center for Chronic Disease Prevention and Health Promotion, 2012), yet very little is know about the psychological consequences of this behavior. The present study suggests that this pattern of consumption is capable of producing

enduring affective disturbances during abstinence, consistent with those seen in chronic alcoholism. It therefore seems reasonable to speculate that binge drinking causes similar changes to extended amygdala structures, mediated by dysregulation of the HPA axis and CNS hyperexcitability.

Conclusion

In summary, the present study shows that voluntary binge drinking in an animal model is capable of producing long-lasting, residual emotional effects after as little as 6 weeks of alcohol exposure. The data provide strong evidence for increased anxiety during withdrawal from binge drinking and modestly support effects of binge drinking on the manifestation of depressive symptoms. These effects show a rapid onset and persist into protracted withdrawal. These affective changes are similar to those seen during withdrawal from chronic exposure in alcohol-dependent animals and likely share similar underlying mechanisms. Further investigation is warranted to better understand the psychological and physiological consequences of binge drinking and how this pattern of behavior may contribute to the transition to addiction.
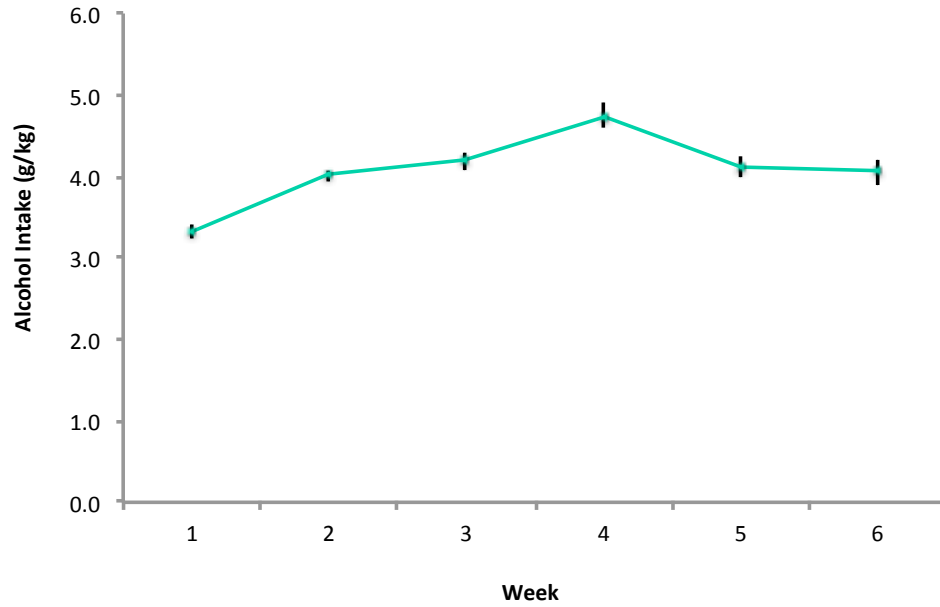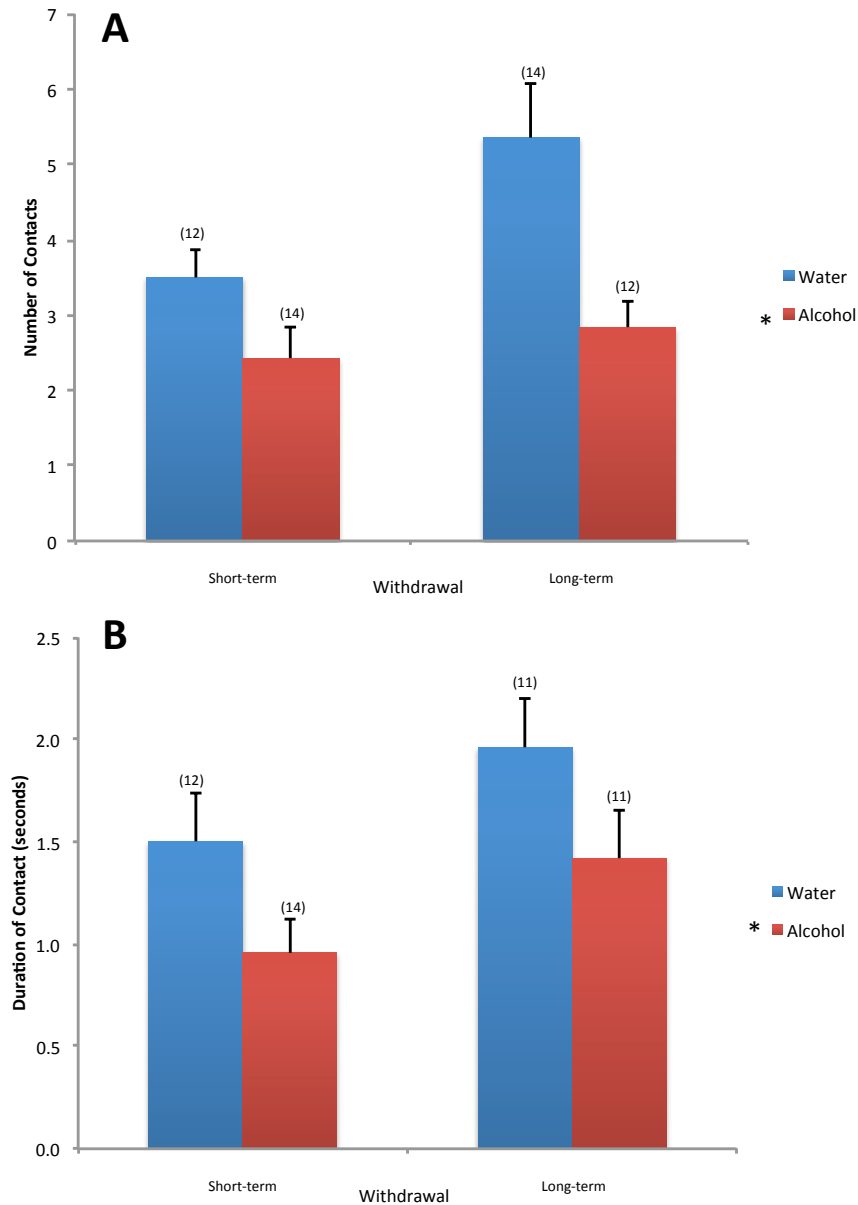
Figure 1. **The DID model of binge drinking yields high alcohol intake with low variability.** B6 mice drinking under modified DID procedures exhibited consistently high levels of alcohol intake across the 6 weeks of drinking. The average alcohol intake of the mice was 4.0±0.06 g/kg/ 2h. Data represent mean ± SEM.

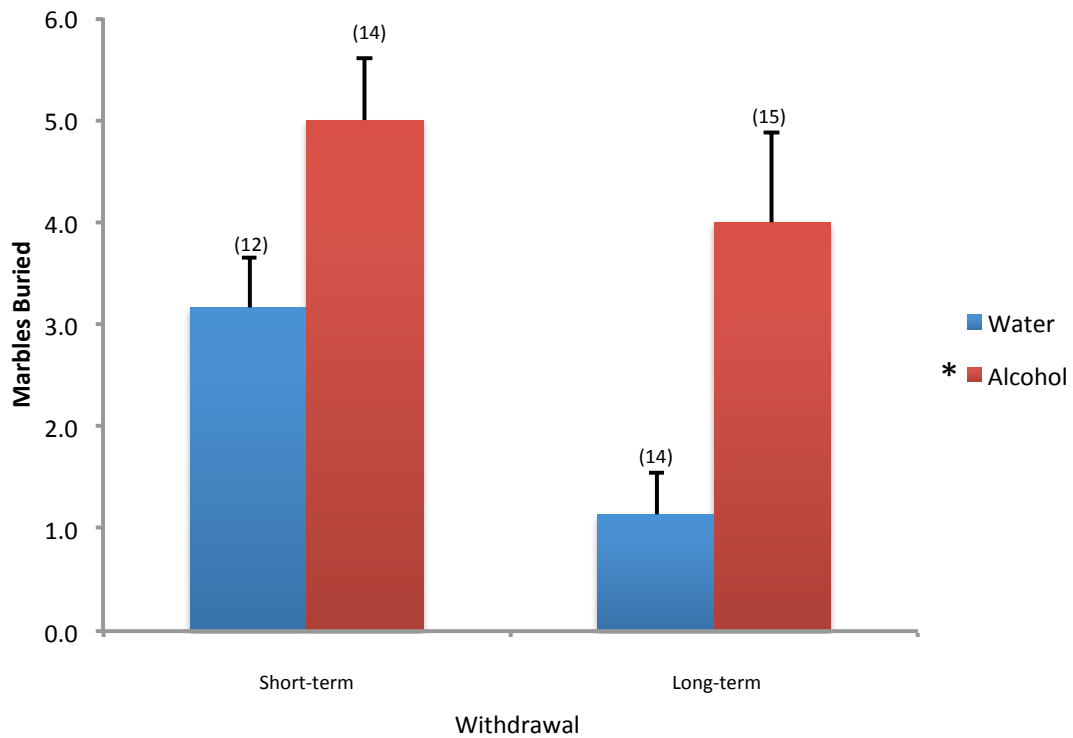*Figure 2*. **Withdrawal from binge drinking increases anxiety in response to novelty.** *(A)* When placed in an activity chamber with a novel object, mice with a history of binge drinking made fewer contacts with the object compared to alcohol-naïve mice. *(B)* alcohol-drinking mice spent less time interacting with the novel object compared to water-drinking controls during the 2-minuute novel object encounter. Data represent mean ± SEM of the number of animals indicated in parentheses, *denotes main group effect $p < 0.05$.
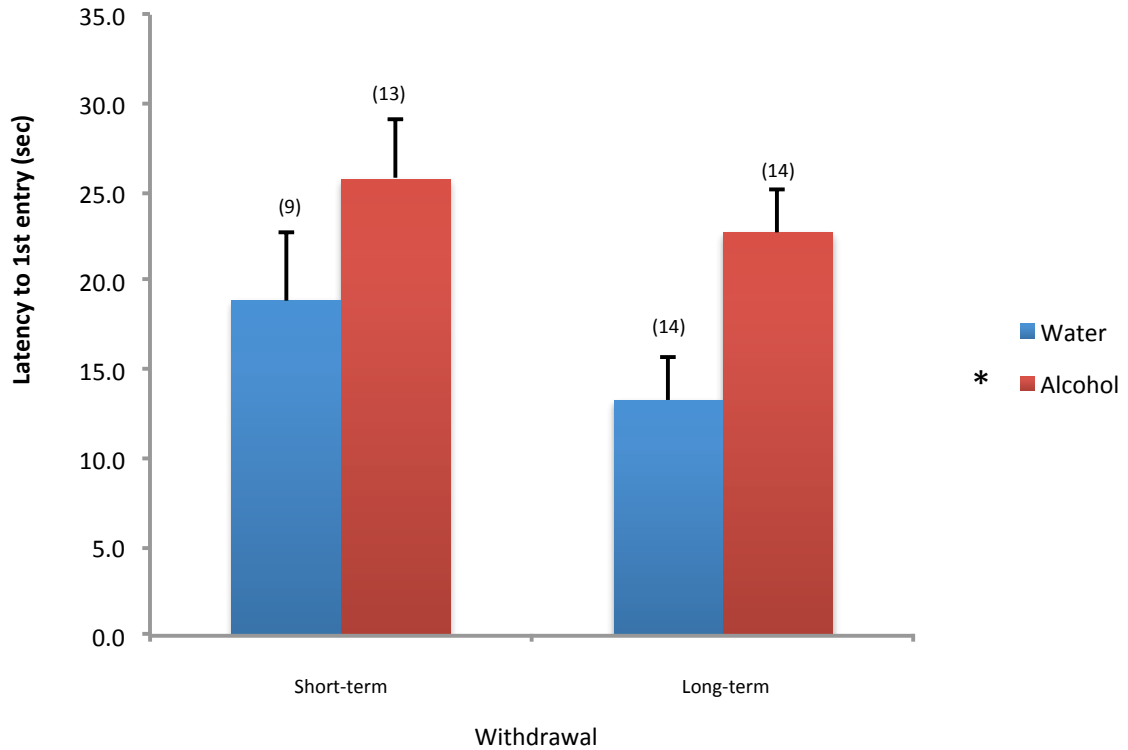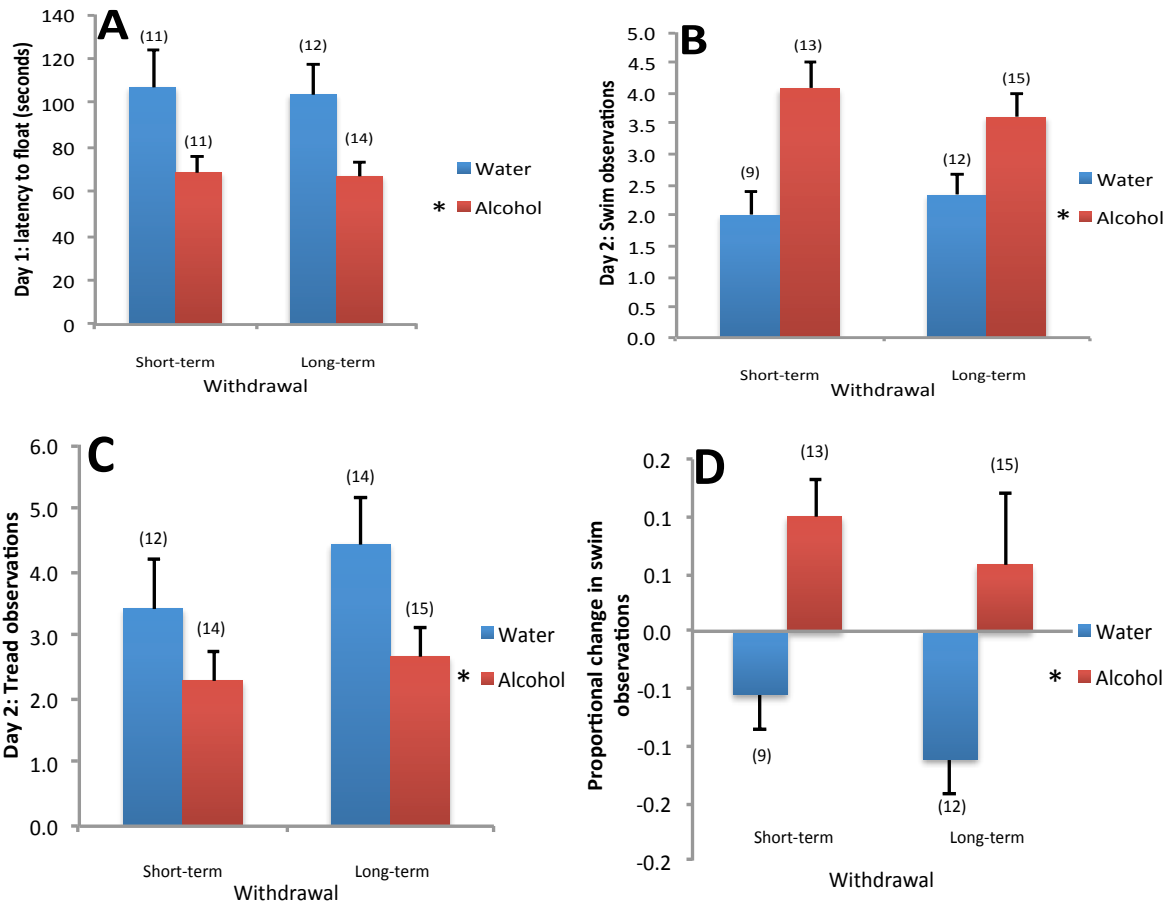
Figure 3. **Withdrawal from binge drinking increases anxiety on the elevated plus maze.**
When tested on an elevated plus-maze, mice with a history of binge drinking had a longer
latency (in sec) to first enter into the open-arm, compared to water-drinking controls. Data
represent mean ± SEM of the number of animals indicated in parentheses, *denotes main
group effect $p < 0.05$.

Figure 4. **Withdrawal from binge drinking increases anxiety-induced defensive burying.**

When tested in a marble burying test, mice with a history of binge drinking buried more

marbles than water-drinking controls during the 20-minute session. Data represent

mean ± SEM of the number of animals indicated in parentheses, [*] denotes main group effect

$p < 0.05$.

Figure 5. **Withdrawal from binge drinking produces mixed effects on behavioral despair.** *(A)* When allowed to swim for 15 min in a Porsolt swim test, mice with a history of binge drinking displayed a shorter latency to first float, compared to water-drinking controls. When tested 24 hours later on a 5-min re-exposure test, mice with a history of binge drinking exhibited *(B)* more swimming behavior and *(C)* less treading behavior. *(D)* Mice with a binge drinking history also exhibited a significant increase in the proportion of swim observations between the 2 swim tests, while water-drinking mice displayed a test-dependent reduction in the proportion of swim observations. Data represent mean ± SEM of the number of animals indicated in parentheses, [*]denotes main group effect $p < 0.05$.
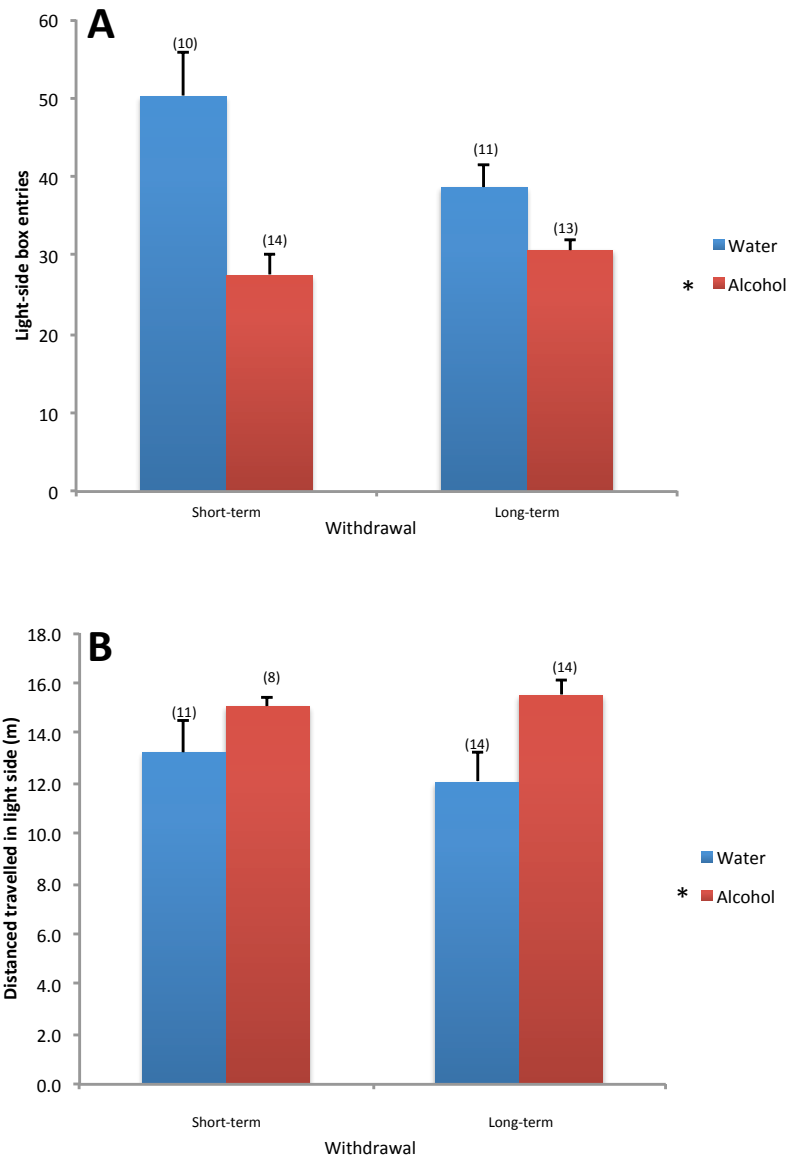
Figure 6. **Withdrawal from binge drinking affects behavior in light/dark shuttle box test.** When tested in a light/dark shuttle box, *(A)* mice with a history of binge drinking made fewer light-side box entries, compared to water-drinking controls during short-term withdrawal. *(B)* Overall, the alcohol-drinking mice showed a greater distance travelled in the light side compared to water-drinking mice. Data represent mean ± SEM of the number of animals indicated in parentheses, [*]denotes main group effect $p < 0.05$.

References

Alheid, G. F. (2003). Extended amygdala and basal forebrain. *Ann N Y Acad Sci, 985*, 185-205.

Alheid, G. F., & Heimer, L. (1988). New Perspectives in Basal Forebrain Organization of Special Relevance for Neuropsychiatric Disorders - the Striatopallidal, Amygdaloid, and Corticopetal Components of Substantia Innominata. *Neuroscience, 27*(1), 1-39. doi: Doi 10.1016/0306-4522(88)90217-5

Bayard, M., McIntyre, J., Hill, K. R., & Woodside, J., Jr. (2004). Alcohol withdrawal syndrome. *Am Fam Physician, 69*(6), 1443-1450.

Becker, H. C. (2000). Animal models of alcohol withdrawal. *Alcohol Research & Health, 24*(2), 105-+.

Briones, T. L., & Woods, J. (2013). Chronic binge-like alcohol consumption in adolescence causes depression-like symptoms possibly mediated by the effects of BDNF on neurogenesis. *Neuroscience, 254*, 324-334. doi: 10.1016/j.neuroscience.2013.09.031

Cloninger, C. R. (1987). Neurogenetic adaptive mechanisms in alcoholism. *Science, 236*(4800), 410-416.

Cozzoli, D. K., Courson, J., Caruana, A. L., Miller, B. W., Greentree, D. I., Thompson, A. B., . . . Szumlinski, K. K. (2012). Nucleus accumbens mGluR5-associated signaling regulates binge alcohol drinking under drinking-in-the-dark procedures. *Alcohol Clin Exp Res, 36*(9), 1623-1633. doi: 10.1111/j.1530-0277.2012.01776.x

Cozzoli, D. K., Courson, J., Wroten, M. G., Greentree, D. I., Lum, E. N., Campbell, R. R., . . . Szumlinski, K. K. (2014). Binge alcohol drinking by mice requires intact group1 metabotropic glutamate receptor signaling within the central nucleus of the amygdale. *Neuropsychopharmacology, 39*(2), 435-444. doi: 10.1038/npp.2013.214

Cozzoli, D. K., Goulding, S. P., Zhang, P. W., Xiao, B., Hu, J. H., Ary, A. W., . . . Szumlinski, K. K. (2009). Binge drinking upregulates accumbens mGluR5-Homer2-PI3K signaling: functional implications for alcoholism. *J Neurosci, 29*(27), 8655-8668. doi: 10.1523/JNEUROSCI.5900-08.2009

Crabbe, J. C., Harris, R. A., & Koob, G. F. (2011). Preclinical studies of alcohol binge drinking. *Ann N Y Acad Sci, 1216*, 24-40. doi: 10.1111/j.1749-6632.2010.05895.x

Crabbe, J. C., Metten, P., Rhodes, J. S., Yu, C. H., Brown, L. L., Phillips, T. J., & Finn, D. A. (2009). A Line of Mice Selected for High Blood Ethanol Concentrations Shows Drinking in the Dark to Intoxication. *Biological Psychiatry, 65*(8), 662-670. doi: Doi 10.1016/J.Biopsych.2008.11.002

Crawley, J., & Goodwin, F. K. (1980). Preliminary-Report of a Simple Animal Behavior Model for the Anxiolytic Effects of Benzodiazepines. *Pharmacology Biochemistry and Behavior, 13*(2), 167-170. doi: Doi 10.1016/0091-3057(80)90067-2

Doremus, T. L., Brunell, S. C., Varlinskaya, E. I., & Spear, L. P. (2003). Anxiogenic effects during withdrawal from acute ethanol in adolescent and adult rats. *Pharmacol Biochem Behav, 75*(2), 411-418.

Driessen, M., Meier, S., Hill, A., Wetterling, T., Lange, W., & Junghanns, K. (2001). The course of anxiety, depression and drinking behaviours after completed detoxification in alcoholics with and without comorbid anxiety and depressive disorders. *Alcohol Alcohol, 36*(3), 249-255.

Egli, M. (2005). Can experimental paradigms and animal models be used to discover clinically effective medications for alcoholism? *Addict Biol, 10*(4), 309-319. doi: 10.1080/13556210500314550

Esel, E. (2006). Neurobiology of alcohol withdrawal inhibitory and excitatory neurotransmitters. *Turk Psikiyatri Derg, 17*(2), 129-137.

Freeman, K., Staehle, M. M., Vadigepalli, R., Gonye, G. E., Ogunnaike, B. A., Hoek, J. B., & Schwaber, J. S. (2013). Coordinated Dynamic Gene Expression Changes in the Central Nucleus of the Amygdala During Alcohol Withdrawal. *Alcoholism-Clinical and Experimental Research, 37*, E88-E100. doi: Doi 10.1111/J.1530-0277.2012.01910.X

Freund, G. (1975). Induction of physical dependence on alcohol in rodents. *Adv Exp Med Biol, 56*, 311-325.

Gosnell, B. A., & Krahn, D. D. (1992). The relationship between saccharin and alcohol intake in rats. *Alcohol, 9*(3), 203-206.

Hall, W., & Zador, D. (1997). The alcohol withdrawal syndrome. *Lancet, 349*(9069), 1897-1900. doi: 10.1016/S0140-6736(97)04572-8

Hascoet, M., & Bourin, M. (2009). The Mouse Light-Dark Box Test. *Mood and Anxiety Related Phenotypes in Mice: Characterization Using Behavioral Tests, 42*, 197-223. doi: Doi 10.1007/978-1-60761-303-9_11

Hasin, D. S., Stinson, F. S., Ogburn, E., & Grant, B. F. (2007). Prevalence, correlates, disability, and comorbidity of DSM-IV alcohol abuse and dependence in the United States: results from the National Epidemiologic Survey on Alcohol and Related Conditions. *Arch Gen Psychiatry, 64*(7), 830-842. doi: 10.1001/archpsyc.64.7.830

Herdegen, T., & Leah, J. D. (1998). Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. *Brain Res Brain Res Rev, 28*(3), 370-490.

Hitzemann, R. (2000). Animal models of psychiatric disorders and their relevance to alcoholism. *Alcohol Res Health, 24*(3), 149-158.

Hughes, J. R. (2009). Alcohol withdrawal seizures. *Epilepsy Behav, 15*(2), 92-97. doi: 10.1016/j.yebeh.2009.02.037

Jung, M. E., Wallis, C. J., Gatch, M. B., & Lal, H. (2000). Abecarnil and alprazolam reverse anxiety-like behaviors induced by ethanol withdrawal. *Alcohol, 21*(2), 161-168.

Kaczmarek, L., & Robertson, H. J. (2002). *Immediate early genes and inducible transcription factors in mapping of the central nervous system function and dysfunction*. Amsterdam Oxford: Elsevier.

Kampov-Polevoy, A., Garbutt, J. C., & Janowsky, D. (1997). Evidence of preference for a high-concentration sucrose solution in alcoholic men. *Am J Psychiatry, 154*(2), 269-270.

Karl, T., Pabst, R., & von Horsten, S. (2003). Behavioral phenotyping of mice in pharmacological and toxicological research. *Experimental and Toxicologic Pathology, 55*(1), 69-83. doi: Doi 10.1078/0940-2993-00301

Katz, R. J. (1982). Animal model of depression: pharmacological sensitivity of a hedonic deficit. *Pharmacol Biochem Behav, 16*(6), 965-968.

Kirchhoff, A. M., & Chester, J. A. (2013). Assessment of Alcohol Withdrawal-Related Behaviors in Animal Models of Alcohol Dependence and Co-Morbid Disorders. *Alcoholism-Clinical and Experimental Research, 37*, 236A-236A.

Kliethermes, C. L. (2005). Anxiety-like behaviors following chronic ethanol exposure. *Neurosci Biobehav Rev, 28*(8), 837-850. doi: 10.1016/j.neubiorev.2004.11.001

Knapp, D. J., Saiers, J. A., & Pohorecky, L. A. (1993). Observations of novel behaviors as indices of ethanol withdrawal-induced anxiety. *Alcohol Alcohol Suppl, 2*, 489-493.

Knight, J. R., Wechsler, H., Kuo, M., Seibring, M., Weitzman, E. R., & Schuckit, M. A.
(2002). Alcohol abuse and dependence among U.S. college students. *J Stud Alcohol,*
63(3), 263-270.

Koob, G. F. (1999). The role of the striatopallidal and extended amygdala systems in drug
addiction. *Ann N Y Acad Sci, 877*, 445-460.

Koob, G. F. (2009). Neurobiological substrates for the dark side of compulsivity in addiction.
Neuropharmacology, 56, 18-31. doi: Doi 10.1016/J.Neuropharm.2008.07.043

Koob, G. F., & Le Moal, M. (1997). Drug abuse: hedonic homeostatic dysregulation.
Science, 278(5335), 52-58.

Koob, G. F., & Le Moal, M. (2001). Drug addiction, dysregulation of reward, and allostasis.
Neuropsychopharmacology, 24(2), 97-129. doi: 10.1016/S0893-133X(00)00195-0

Kranzler, H. R., Sandstrom, K. A., & Van Kirk, J. (2001). Sweet taste preference as a risk
factor for alcohol dependence. *Am J Psychiatry, 158*(5), 813-815.

Kushner, M. G., Abrams, K., & Borchardt, C. (2000). The relationship between anxiety
disorders and alcohol use disorders: a review of major perspectives and findings. *Clin*
Psychol Rev, 20(2), 149-171.

Linnoila, M., Mefford, I., Nutt, D., & Adinoff, B. (1987). NIH conference. Alcohol
withdrawal and noradrenergic function. *Ann Intern Med, 107*(6), 875-889.

Markou, A., & Koob, G. F. (1991). Postcocaine anhedonia. An animal model of cocaine
withdrawal. *Neuropsychopharmacology, 4*(1), 17-26.

McBride, W. J., Kimpel, M. W., Schultz, J. A., McClintick, J. N., Edenberg, H. J., & Bell, R.
L. (2010). Changes in gene expression in regions of the extended amygdala of
alcohol-preferring rats after binge-like alcohol drinking. *Alcohol, 44*(2), 171-183. doi:
10.1016/j.alcohol.2009.12.001

McMillen, B. A. (1997). Toward a definition of a valid model of alcoholism: multiple animal models for multiple diseases. *Alcohol, 14*(4), 409-419.

Misslin, R., & Ropartz, P. (1981). Responses in Mice to a Novel Object. *Behaviour, 78*, 169-177. doi: Doi 10.1163/156853981x00301

Moore, E. M., & Boehm, S. L. (2009). Site-Specific Microinjection of Baclofen Into the Anterior Ventral Tegmental Area Reduces Binge-Like Ethanol Intake in Male C57BL/6J Mice. *Behavioral Neuroscience, 123*(3), 555-563. doi: Doi 10.1037/A0015345

National Center for Chronic Disease Prevention and Health Promotion. (2012). *Binge drinking: Nationwide problem, local solutions.* .

National Institute on Alcohol Abuse and Alcoholism. (2004). NIAAA Council Approves Definition of Binge Drinking *NIAAA Newsletter* (Vol. 3, pp. 3).

Njunge, K., & Handley, S. L. (1991). Evaluation of Marble-Burying Behavior as a Model of Anxiety. *Pharmacology Biochemistry and Behavior, 38*(1), 63-67. doi: Doi 10.1016/0091-3057(91)90590-X

Onaivi, E. S., Todd, S., & Martin, B. R. (1989). Behavioral effects in the mouse during and following withdrawal from ethanol ingestion and/or nicotine administration. *Drug Alcohol Depend, 24*(3), 205-211.

Paxinos, G., & Franklin, K. (2004). *The Mouse Brain in Stereotaxic Coordinates* (2nd ed.). San Diego, CA: Elsevier Academic Press.

Piazza, P. V., & Le Moal, M. L. (1996). Pathophysiological basis of vulnerability to drug abuse: role of an interaction between stress, glucocorticoids, and dopaminergic neurons. *Annu Rev Pharmacol Toxicol, 36*, 359-378. doi: 10.1146/annurev.pa.36.040196.002043

Porsolt, R. D., Bertin, A., & Jalfre, M. (1977). Behavioral despair in mice: a primary

screening test for antidepressants. *Arch Int Pharmacodyn Ther, 229*(2), 327-336.

Quintino-dos-Santos, J. W., Muller, C. J., Santos, A. M., Tufik, S., Rosa, C. A., &

Schenberg, L. C. (2014). Long-lasting marked inhibition of periaqueductal gray-

evoked defensive behaviors in inescapably-shocked rats. *Eur J Neurosci, 39*(2), 275-

286. doi: 10.1111/ejn.12410

Rasmussen, D. D., Boldt, B. M., Bryant, C. A., Mitton, D. R., Larsen, S. A., & Wilkinson, C.

W. (2000). Chronic daily ethanol and withdrawal: 1. Long-term changes in the

hypothalamo-pituitary-adrenal axis. *Alcohol Clin Exp Res, 24*(12), 1836-1849.

Rasmussen, D. D., Mitton, D. R., Green, J., & Puchalski, S. (2001). Chronic daily ethanol

and withdrawal: 2. Behavioral changes during prolonged abstinence. *Alcohol Clin

Exp Res, 25*(7), 999-1005.

Rassnick, S., Heinrichs, S. C., Britton, K. T., & Koob, G. F. (1993). Microinjection of a

Corticotropin-Releasing Factor Antagonist into the Central Nucleus of the Amygdala

Reverses Anxiogenic-Like Effects of Ethanol Withdrawal. *Brain Research, 605*(1),

25-32. doi: Doi 10.1016/0006-8993(93)91352-S

Rhodes, J. S., Best, K., Belknap, J. K., Finn, D. A., & Crabbe, J. C. (2005). Evaluation of a

simple model of ethanol drinking to intoxication in C57BL/6J mice. *Physiol Behav,

84*(1), 53-63. doi: 10.1016/j.physbeh.2004.10.007

Roberto, M., Gilpin, N. W., & Siggins, G. R. (2012). The central amygdala and alcohol: role

of gamma-aminobutyric acid, glutamate, and neuropeptides. *Cold Spring Harb

Perspect Med, 2*(12), a012195. doi: 10.1101/cshperspect.a012195

Sadock, B. J., Sadock, V. A., Ruiz, P., & Kaplan, H. I. (2009). *Kaplan & Sadock's comprehensive textbook of psychiatry* (9th ed.). Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins.

Saitz, R., & O'Malley, S. S. (1997). Pharmacotherapies for alcohol abuse. Withdrawal and treatment. *Med Clin North Am, 81*(4), 881-907.

Smith, R. J., & Aston-Jones, G. (2008). Noradrenergic transmission in the extended amygdala: role in increased drug-seeking and relapse during protracted drug abstinence. *Brain Struct Funct, 213*(1-2), 43-61. doi: 10.1007/s00429-008-0191-3

Sobin, C., & Sackeim, H. A. (1997). Psychomotor symptoms of depression. *Am J Psychiatry, 154*(1), 4-17.

Spanagel, R. (2000). Recent animal models of alcoholism. *Alcohol Res Health, 24*(2), 124-131.

Stephens, M. A., & Wand, G. (2012). Stress and the HPA axis: role of glucocorticoids in alcohol dependence. *Alcohol Res, 34*(4), 468-483.

Stewart, R. B., Russell, R. N., Lumeng, L., Li, T. K., & Murphy, J. M. (1994). Consumption of sweet, salty, sour, and bitter solutions by selectively bred alcohol-preferring and alcohol-nonpreferring lines of rats. *Alcohol Clin Exp Res, 18*(2), 375-381.

Su, Z. I., Wenzel, J., Ettenberg, A., & Ben-Shahar, O. (2013). Prior extended daily access to cocaine elevates the reward threshold in a conditioned place preference test. *Addict Biol*. doi: 10.1111/adb.12053

Substance Abuse and Mental Health Services Administration. (2011). *Results from the 2010 National Survey on Drug Use and Health: Summary of National Findings*. Rockville, MD.

Szumlinski, K. K., Diab, M. E., Friedman, R., Henze, L. M., Lominac, K. D., & Bowers, M. S. (2007). Accumbens neurochemical adaptations produced by binge-like alcohol consumption. *Psychopharmacology (Berl), 190*(4), 415-431. doi: 10.1007/s00213-006-0641-7

Walf, A. A., & Frye, C. A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nature Protocols, 2*(2), 322-328. doi: Doi 10.1038/Nprot.2007.44

Weiss, F., Parsons, L. H., Schulteis, G., Hyytia, P., Lorang, M. T., Bloom, F. E., & Koob, G. F. (1996). Ethanol self-administration restores withdrawal-associated deficiencies in accumbal dopamine and 5-hydroxytryptamine release in dependent rats. *Journal of Neuroscience, 16*(10), 3474-3485.