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Binge alcohol drinking elicits persistent negative affect in mice

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HIGHLIGHTS

- We studied the affective consequences of withdrawal from binge drinking in male mice.
- Binge drinking elicited signs of anxiety and depression during both short and protracted withdrawal.
- Binge drinking elevated cellular activity within extended amygdala brain structures.
- Withdrawal from chronic binge drinking elicits a persistent dysphoric state that correlates with hyperactivity within the extended amygdala.

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ABSTRACT

Cessation from chronic alcohol abuse often produces a dysphoric state that can persist into protracted withdrawal. This dysphoric state is theorized to function as a negative reinforcer that maintains excessive alcohol consumption and/or precipitates relapse in those struggling to abstain from alcohol. However, we know relatively little regarding the impact of cessation from binge drinking on behavioral measures of negative affect and related neurobiology. Male C57BL/6J mice were given access to unsweetened 20% alcohol for 6 weeks under modified Drinking-in-the-dark procedures, followed by behavioral testing beginning either 1 or 21 days into withdrawal. Mice were administered a behavioral test battery consisting of: the elevated plus maze, light/dark box, novel object test, marble burying test, Porsolt forced swim test and sucrose preference test to assess anxiogenic and depressive signs. Egr1 immunostaining was used to quantify cellular activity within the central nucleus of the amygdala (CEA), basolateral amygdala (BLA), bed nucleus of the stria terminalis (BNST), and the nucleus accumbens (Acb) shell (AcbSh) and core (AcbC). Compared to water controls, alcohol-drinking mice exhibited higher indices of emotionality in the majority of behavioral assays. The hyper-emotionality exhibited by binge drinking mice was apparent at both withdrawal time-points and correlated with higher Egr1+ cell counts in the CEA and BNST, compared to controls. These data show that affective symptoms emerge very early after cessation of binge drinking and persist into protracted withdrawal. A history of binge drinking is capable of producing enduring neuroadaptations within brain circuits mediating emotional arousal.

1. 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 [1]. Binge drinking is defined as a pattern of alcohol intake sufficient to produce a blood alcohol concentration (BAC) of ≥80 mg% in a 2-h period [2]. Repeated bouts of 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 [3]. Frequent binge drinking is also a significant risk factor for the development of alcoholism [4]. As such, characterizing the neurobiological impact of binge drinking is crucial for understanding the consequences of this pattern of excessive alcohol consumption, which will aid in the development of pharmacotherapies for this prevalent form of alcohol-use disorder.

The elevated anxiety and depression during alcohol withdrawal is an aversive state frequently reported in the human population [5] that is theorized to be a compelling source of negative reinforcement fueling compulsive drug-seeking behavior and relapse [6]. However, confounding subject factors render it difficult to discern whether or not binge-drinking history is a sufficient antecedent to a negative affect state in humans. Moreover, it is impossible to

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study the cellular and molecular mechanisms underpinning emotional disturbances during alcohol withdrawal in a systematic, controlled, manner through studies of humans. As such, we have gained the majority of our insight into the psychobiological impact of alcohol from studies of animal models of alcohol use disorders. This being said, there exists limited literature describing emotional anomalies during withdrawal in animal models of alcohol abuse; these studies have typically relied on either non-contingent alcohol delivery paradigms (e.g., vapor inhalation, injection, or gavage) or contingent models based prolonged access to adulterated alcohol solutions (e.g., liquid diet) [7]. While effective at producing physical dependence and robust behavioral indices of negative affect, such models arguably lack face validity and often involve higher alcohol doses than animals would consume voluntarily [8–11]. Given the gaps in our extant knowledge concerning the emotional consequences of binge drinking, herein, we characterized the effects of voluntary alcohol intake on behavioral measures of anxiety and depression within the context of a modified version of the well-established "Drinking in the dark" (DID) murine model of binge drinking [e.g.,12].

Emotional dysregulation during alcohol withdrawal is largely attributed to changes within the extended amygdala [e.g.,7]. The extended amygdala is a basal forebrain macrosystem that acts as a subcortical relay station between the brainstem, thalamus, and cortical areas and includes the bed nucleus of the stria terminalis (BNST), the shell of the nucleus accumbens (AcbSh), and the central nucleus of the amygdala (CEA) [13]. These areas are heavily implicated in the processing of anxiety, fear, depression, reward, and reinforcement and alcohol-induced changes within this macrosystem are implicated in the emotional disturbances that can occur during alcohol abstinence [14]. As for withdrawal-induced negative affect (see above), the vast majority of our understanding of how alcohol withdrawal impacts extended amygdala function of relevance to emotional dysregulation has been derived from studies employing non-contingent alcohol administration or the use of adulterated alcohol diets [7]. However, consistent with this prior work, a dysregulation of extended amygdala function is reported during alcohol withdrawal in animal models of binge drinking. For instance, increased indices of glutamate transmission within extended amygdala structures are observed at 24 h following a month-long history of binge drinking [15,16], while changes in the expression of genes related to synaptic transmission and neuronal plasticity occur within the AcbSh and CeA of binging rodents during early withdrawal (i.e., 1–6 h post-binge) [17,18]. Thus, binge alcohol drinking appears to induce neuroadaptations within the extended amygdala similar to those reported in traditional models of alcohol dependence [19]. However, to the best of our knowledge, no study to date has related binge drinking-induced changes in extended amygdala function to the manifestation of negative affect during withdrawal from binge drinking. Given the gaps in the extant literature, we conducted an immunohistochemical analysis of the expression of the transcription factor Egr-1 within the extended amygdala and adjacent structures to correlate cellular activity [20] with the manifestation of anxiety and depressive behavior during withdrawal from binge drinking. We hypothesized that a history of binge drinking would augment behavioral indices of negative affect and that withdrawal-induced negative affect would be associated with increased cellular activity within the extended amygdala.

2. Methods

2.1. Subjects

This study used 60 adult C57BL/6J (B6) male mice that were 8 weeks of age at onset of drinking and weighed 25–30 g (Jackson Laboratories, Sacramento, CA). Animals were randomly divided into an alcohol-drinking group ($n = 30$; hereafter referred to as Alcohol mice) and a water-drinking group ($n = 30$; hereafter referred to as Water mice) and then individually housed in standard, Plexiglas cages, under a 12-h-reverse light/dark cycle (lights off at 10am), in a temperature-controlled vivarium (23 °C). Food and water were available ad libitum, with the exception of the 2-h alcohol drinking period, during which time the home cage water bottle was removed. 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 2010) and approved by the IACUC of the University of California, Santa Barbara.

2.2. Drinking-in-the dark (DID) procedures

To elicit consistently high alcohol consumption, we employed a modified version of the DID model, which results in alcohol intakes between 3.5–5.0 g/kg alcohol in a 2-h period and yields blood alcohol concentrations (BACs) in excess of 80 mg% [e.g.,12]. 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 mice were allowed to drink for 2 h, at which point the alcohol bottles were removed and the home cage water bottles were replaced. Control animals received an identical sipper tube of filtered tap water *in lieu* of alcohol. For practical reasons, mice were subjected to these drinking conditions 5 days per week (M–F) over the course of 6 weeks (total drinking days = 30). Each day, the amount of alcohol consumed was calculated by bottle weight immediately before and after the drinking period. Unfortunately, technical difficulties with our Analox Analyzer precluded our ability to determine BACs in this study. Thus, BACs were estimated from observed intakes and based on the results of published correlational analyses in B6 mice [12,21,22], as conducted previously [23].

2.3. Behavioral testing

We administered a 2-day test battery to assay for alcohol withdrawal-induced changes in behavior in both the short-term and long-term (respectively 1–2 vs. 21–22 days following last alcohol presentation; $n = 15$/group/withdrawal time-point). At both time-points, testing for affect 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 first in a light/dark shuttle box or novel object encounter (order randomized across cohorts), followed by a 15-min swim test. The 15-min swim test occurred at the end of the first day to allow mice time to recuperate, as per our IACUC's request. On the second test day, mice were tested first on the elevated plus maze or for marble burying (order randomized across cohorts), followed by a 5-min swim retest and animals were sacrificed and brain tissue was harvested immediately upon completion of the swim retest. All tests were conducted under standard ambient lighting and the details of the procedures employed for each of these paradigms are provided below.

2.3.1. 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 [24] and thus, we examined for the effects of early vs. late withdrawal from binge drinking on sucrose preference in our mice. For this, animals were given overnight access to 2 identical sipper tubes, one contained 5% sucrose and the other contained tap water. The bottles were weighed prior to being placed on the home cage at 17:00 h. Sixteen hours later (09:00 h the next day), the bot-