The histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) alleviates depression-like behavior and normalizes epigenetic changes in the hippocampus during ethanol withdrawal

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Abstract

Withdrawal from chronic alcohol drinking can cause depression, leading to an inability to function in daily life and an increased risk for relapse to harmful drinking. Understanding the causes of alcohol withdrawal-related depression may lead to new therapeutic targets for treatment. Epigenetic factors have recently emerged as important contributors to both depression and alcohol use disorder (AUD). Specifically, acetylation of the N-terminal tails of histone proteins that package DNA into nucleosomes is altered in stress-induced models of depression and during alcohol withdrawal. The goal of this study was to examine depression-like behavior during alcohol withdrawal and associated changes in histone acetylation and expression of histone deacetylase 2 (HDAC2) in the hippocampus, a brain region critical for mood regulation and depression. Male Sprague-Dawley rats were treated with the Lieber-DeCarli ethanol liquid diet for 15 days and then underwent withdrawal. Rats were treated with the HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA), during withdrawal and were tested for depression-like behavior. In a separate group of rats, the hippocampus was analyzed for mRNA and protein expression of HDAC2 and levels of histone H3 lysine 9 acetylation (H3K9ac) during chronic ethanol exposure and withdrawal. Rats undergoing ethanol withdrawal exhibited depression-like behavior and had increased HDAC2 and decreased H3K9ac levels in specific structures of the hippocampus. Treatment with SAHA during withdrawal ameliorated depression-like behavior and normalized changes in hippocampal HDAC2 and H3K9ac levels. These results demonstrate that ethanol withdrawal causes an altered epigenetic state in the hippocampus. Treatment with an HDAC inhibitor can correct this state and alleviate depression-like symptoms developed during withdrawal. Targeting histone acetylation may be a novel strategy to reduce ethanol withdrawal-induced depression.

Introduction

Alcohol use disorder (AUD) is highly comorbid with depression (Boden & Fergusson, 2011). One reason for this comorbidity is that abstinence from chronic alcohol drinking can cause depression (Petit, Luminet, Cordovil de Sousa Uva, Monhonval, et al., 2017; Petit, Luminet, Cordovil de Sousa Uva, Zorbas, et al., 2017). The functional link between AUD and depression is supported by preclinical studies demonstrating increased depression-like behavior in rodents during alcohol withdrawal (Antón et al., 2017; Geatchew, Hauser, Csoka, Taylor, & Tizabi, 2017; Kang, Li, Bekker, & Ye, 2018; Li et al., 2017; Pang, Renoir, Du, Lawrence, & Hannan, 2013; Roni & Rahman, 2017; Walker et al., 2010; Yawalkar, Changotra, & Gupta, 2018). Depression during abstinence from alcohol also contributes to relapse to alcohol drinking, thus perpetuating the cycle of addiction (Greenfield et al., 1998; Oliva et al., 2018). Depression and alcohol abuse negatively affect overall health, productivity, and quality of life. A better understanding of the brain mechanisms contributing to alcohol withdrawal-induced depression would be
extremely helpful in the development of novel therapeutic approaches effective in reducing comorbid alcohol abuse and depression.

The hippocampus is an important region of the brain for modulating emotional states such as depression (Akil et al., 2018; Fanselow & Dong, 2010). Chronic alcohol consumption is associated with decreased hippocampal volume, as measured using magnetic resonance imaging in alcohol-dependent subjects compared with controls (Beresford et al., 2006). Notably, this is also observed in patients with major depressive disorder (Malykhin, Carter, Seres, & Coupland, 2010), suggesting a link between alcohol-induced changes in the hippocampus and depression. Numerous molecules, signal transduction pathways, and basic cellular processes in the hippocampus are causally linked with depression. These include alterations in the serotonergic system, brain-derived neurotrophic factor (BDNF) expression, cyclic AMP response element binding protein (CREB) activity, neurogenesis, and neuroinflammation (Kubera, Obuchowicz, Goehler, Brzeszcz, & Maes, 2011; Rogers, Renoir, & Hannan, 2017).

Epigenetic mechanisms, such as acetylation of the N-terminal tails of histone proteins that package DNA into nucleosomes, also play an important role in depression (Misztak, Panczyszyn-Trzewik, & Sowa-Łotma, 2018). Levels of histone acetylation and the histone deacetylases (HDACs) that remove acetyl groups from the histone tails and thus decrease chromatin accessibility, are altered in the hippocampus of mice and rats in various models of stress-induced depression (Covington, Vialou, LaPlant, Ohnishi, & Nestler, 2011; Ferland & Schrader, 2011; Han, Sung, Chung, & Kwon, 2014; Kvet al., 2018; Liu et al., 2014; Tsankova et al., 2006). Of note, systemic or intra-hippocampal treatment of animals with HDAC inhibitors can reduce depression-like behavior (Covington et al., 2011; Gundersen & Blendy, 2009; Han et al., 2014; Liu et al., 2014; Yamawaki et al., 2012), and injection of MS-275 (Entinostat) directly into the hippocampus of mice subjected to chronic social defeat stress reversed the deficit in sucrose preference and increased social interaction and acetylated histone H3 levels in the hippocampus (Covington et al., 2011).

Similar to stress-induced models of depression, withdrawal from chronic alcohol exposure also has profound effects on histone acetylation and HDAC expression (Pandey, Ugale, Zhang, Tang, & Prakash, 2008; Simon-O’Brien et al., 2015; You, Zhang, Sakhar kar, Teppen, & Pandey, 2014). Decreased histone H3 and H4 acetylation and elevated HDAC activity were observed in the amygdala of rats during withdrawal from chronic alcohol drinking, along with an associated increase in anxiety-like behavior (Pandey et al., 2008; You et al., 2014). Treatment of rats during ethanol withdrawal with the histone deacetylase inhibitor trichostatin A (TSA) attenuated anxiety-like behavior and restored normal levels of histone acetylation in the amygdala (Pandey et al., 2008; You et al., 2014). Additionally, in a rat model of dependence induced by ethanol vapor exposure, treatment with MS-275 decreased drinking only in ethanol-dependent rats and altered histone acetylation in various brain regions (Simon-O’Brien et al., 2015). In mice subjected to repeated ethanol injections and withdrawal, histone H3 lysine 9 acetylation (H3K9ac) was decreased and HDAC2 expression was increased in the ventral tegmental area (Arora et al., 2013). Interestingly, adolescent alcohol exposure leads to an increase in HDAC activity and deficits in H3K9ac in rat hippocampus that are associated with decreased neurogenesis in adulthood. These changes were normalized by TSA treatment (Sakhar kar et al., 2016). Together, these results indicate that alcohol withdrawal leads to dysregulated histone acetylation due to an increase in HDAC2 expression in several brain regions and that treatment with HDAC inhibitors can normalize this imbalance and alleviate negative affective states induced by alcohol withdrawal. However, histone acetylation and HDAC2 expression in the hippocampus and associated depression-like behavior during withdrawal after chronic ethanol exposure have not been examined.

In this study, we hypothesized that withdrawal from chronic alcohol exposure would result in depression-like behavior and lead to a corresponding decrease in histone acetylation associated with an increase in H3K2ac expression in the hippocampus. Therefore, we investigated depression-like behavior and levels of HDAC2 and H3K9ac in the hippocampus during alcohol withdrawal. We also tested whether treatment with the HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA, also known as Vorinostat), would be effective in attenuating depression-like behavior induced by alcohol withdrawal and normalize H3K9ac levels and HDAC2 expression. Our results provide preclinical evidence that using an HDAC inhibitor in individuals with comorbid AUD and depression, particularly alcohol-induced depression, might be an effective novel therapeutic strategy to treat these disorders.

Materials and methods

Animals

Adult male Sprague–Dawley rats (postnatal day 75–85) weighing ~250 g were purchased from Harlan (Indianapolis, Indiana) and individually housed in a temperature-controlled room with a 12/12-h light/dark cycle, with food and water provided ad libitum prior to beginning the chronic ethanol treatment protocol described below. All procedures were conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Illinois at Chicago Institutional Animal Care and Use Committee.

Chronic ethanol treatment

Rats were fed with the nutritionally complete Lieber-DeCarli control liquid diet or ethanol liquid diet (Bio-Serv; Frenchtown, New Jersey) as their only source of food and fluid as previously described (Pandey et al., 2008). Rats were randomly assigned to three treatment groups: i) control-diet fed, ii) ethanol-diet fed (no withdrawal), and iii) ethanol-diet fed with a 24-h withdrawal, referred to as the ethanol-withdrawn group. Briefly, rats were first fed 80 mL/day control diet for 3 days. The control group continued with control diet for 16 days, while the ethanol groups were gradually introduced to ethanol (1.8%-8.1% within 7 days), and then maintained on 9% v/v ethanol diet for 15 days. Ethanol-withdrawn rats were switched to control liquid diet for 24 h after removal of the ethanol liquid diet. One week before behavioral testing (described below), rats were handled for 3 min per day by the same individual to habituate them to handling. Rats were pair fed and their liquid diet intake and body weights were closely monitored. We have previously reported blood ethanol levels in the range of 172–198 mg% for ethanol-diet fed (no withdrawal) rats, whereas the blood ethanol level after 24 h of ethanol withdrawal was undetectable using this alcohol treatment paradigm (Pandey et al., 2008; You et al., 2014).

Drug treatment

Rats in the control and ethanol-withdrawn groups were treated intraperitoneally (i.p.) with either 50 mg/kg SAHA (Selleck Chemicals; Houston, Texas) in 2% DMSO, 40% PEG 300, 5% propylene glycol, and 1% Tween 80 (vehicle) or vehicle only, 2 h before behavioral testing or euthanasia for tissue collection at 24 h of withdrawal. All rats were euthanized by decapitation under iso-flurane anesthesia.

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Sucrose splash test

Depression-like behavior was measured using the splash test as described by Marrocco et al. (2014). A 10% sucrose solution was sprayed on the dorsal coat and abdomen of each rat in a clear cage to induce grooming behavior. The time the rat spent grooming and latency to groom was recorded for a period of 5 min after application of the sucrose solution. This test takes advantage of the natural propensity of the rats to groom, with a reduction in grooming and increased latency to groom indicating anhedonia and a lack of motivation for self-care. Decreased grooming time in the splash test is observed in rats after manipulations that induce multiple indices of depression-like behavior such as prenatal restraint stress, maternal separation, and chronic unpredictable mild stress (Hu et al., 2017; Marrocco et al., 2014; Masrour, Peeri, Azarbayjani, & Hosseini, 2018). Moreover, treatment with antidepressant medications such as fluoxetine normalizes grooming time in the splash test in mice and rats (Marrocco et al., 2014; Masrour et al., 2018; Santarelli et al., 2003; Surget et al., 2008; Yalcin, Belzung, & Surget, 2008).

Sucrose preference test

Bottles containing the control liquid diet were removed from the home cage at 24 h of withdrawal (of both ethanol-withdrawn and control rats) and replaced with two standard water bottles containing either a 0.5% sucrose solution or water. Bottles were weighed after 1 h and sucrose preference was calculated as the volume of sucrose solution consumed divided by the total volume of fluid consumed × 100. Decreased sucrose preference is indicative of anhedonia, a defining symptom of major depressive disorder, and is normalized by chronic treatment with antidepressants (Dichter, Damiano, & Allen, 2012; Papp, Moryl, & Willner, 1996; Willner, Towell, Sampson, Sophokleous, & Muscat, 1987).

In situ reverse transcription-polymerase chain reaction (RT-PCR)

In situ RT-PCR of Hdac2 mRNA was performed on 40-μm-thick coronal brain sections, as described previously (Pandey et al., 2008; Zhang et al., 2010). Briefly, brain sections were treated with proteinase K and then digested with DNase. Sections were rinsed in PBS and transferred to PCR tubes containing 100 μL of PCR reaction mixture (Applied Biosystems; Foster City, California) to reverse transcribe for 1 h at 42 °C with oligo d(T)16 and reverse transcriptase (RT) enzyme. PCR was then performed as follows: 95 °C for 5 min; 95 °C for 30 s, 58 °C for 30 s, 72 °C for 30 s, for a total of 25 cycles; followed by 72 °C for 7 min using Taq DNA polymerase enzyme, 100 pmol each of Hdac2 forward and reverse primers (Hdac2 forward: 5′-CGTTGGCTAGTGCCTG; Hdac2 reverse: 5′-GGCGCTGACTGCTG; MBL International; Woburn, Massachusetts) and 1 mM of each NTP (except that dTTP was replaced by digoxigenin [DIG]-11-dUTP) in 100 μL reaction mixture. Sections were then mounted on slides and Hdac2 mRNA-positive cells were detected using an alkaline phosphatase conjugated anti-DIG antibody with nitro-blue tetrazolium chloride and 5-bromo-4-chloro-3′-indolyl phosphate p-toluidine (NBT-BCIP) as the substrate, yielding a purple reaction product (Roche Molecular Biochemical; Mannheim, Germany). The intensity of Hdac2-positive cell bodies in the cornu ammonis (CA1, CA3, and dentate gyrus [DG]) region of the dorsal hippocampus (approximately −2.3 to −3.3 mm posterior to bregma) was obtained by outlining the cell body layer and measuring the intensity using NIH ImageJ software from each section of 2–3 brain sections from each rat. Values were averaged and presented as mean intensity/100 μm².

Gold immunolabeling of HDAC2 and H3K9ac

HDAC2 and acetylated H3K9ac proteins were measured in the dorsal hippocampus of the rat brain using gold immunolabeling (Pandey et al., 2008; Sakharkar et al., 2014). Free-floating 20-μm-thick coronal sections were incubated with antibodies against HDAC2 (catalog number JM-3602-100, MBL International; Woburn, Massachusetts) and H3K9ac (catalog number 06-942, Millipore; Billerica, Massachusetts). After washing with PBS, the sections were incubated in gold particle-labeled goat anti-rabbit secondary antibodies (Nanoprobes; Yaphank, New York) and were counterstained using the silver enhancement kit (Ted Pella; Redding, California). Gold immunolabeling was quantified using an image analysis system (Loats Associates; Westminster, Maryland) at 100× magnification. We first set up a threshold for counting by outlining an area without staining, and then several object fields in each section were used for counting immunogold particles. Mean counts for each group were calculated and results are presented as the mean (±SEM) of the number of immunogold particles/100 μm² area in the CA1, CA3, and dentate gyrus (DG) regions of the hippocampus.

Statistical analysis

All data are presented as the mean ± SEM. Statistical testing was performed on the in situ RT-PCR and immunogold labeling data with a one-way analysis of variance (ANOVA) within each region of the hippocampus, followed by Tukey’s multiple comparisons test. Behavioral data were analyzed using a two-way ANOVA followed by post hoc comparisons using Sidak’s multiple comparisons tests. Statistical tests were performed using Prism software (GraphPad; San Diego, California, version 7). A value of p < 0.05 was considered to be significant.

Results

Withdrawal from chronic ethanol drinking causes depression-like behavior that is reversed by SAHA treatment

Withdrawal from chronic ethanol exposure is associated with a depression-like state that has been observed behaviorally in mice and rats (Antón et al., 2017; Getachew et al., 2017; Kang et al., 2018; Li et al., 2017; Pang et al., 2013; Roni & Rahman, 2017; Walker et al., 2010; Yawalkar et al., 2018). We examined depression-like behavior using the splash test in rats that were fed with a control or chronic ethanol Lieber-DeCarli diet and underwent 24 h of withdrawal. We also tested for the effect of the HDAC inhibitor SAHA, given acutely 2 h prior to testing, since numerous studies have demonstrated that HDAC inhibitors, including SAHA, can reduce depression-like behavior in rats and mice (reviewed in Misztak et al., 2018). Rats withdrawn from chronic ethanol drinking groomed less and had increased latency to groom in the splash test, indicative of depression-like behavior, while treatment with SAHA normalized grooming time (Fig. 1A and B). A two-way ANOVA indicated a significant withdrawal by SAHA interaction in grooming time [F(1,47) = 8.18, p = 0.006] and latency to groom [F(1,47) = 5.86, p = 0.019], but no main effects of ethanol withdrawal [grooming time: F(1,47) = 0.21, p = 0.65; latency: F(1,47) = 0.51, p = 0.48] or SAHA treatment [grooming time: F(1,47) = 0.00028, p = 0.99; latency: F(1,47) = 2.0, p = 0.16]. Post hoc multiple comparisons tests demonstrated that there was a nearly significant decrease in grooming time and increased latency to groom between control and ethanol-withdrawn rats within the vehicle-treated group (grooming time: p = 0.054; latency: p = 0.072), but there was no difference between control and ethanol-withdrawn rats within the SAHA-treated group (grooming time: p = 0.16; latency: p = 0.39).
Latency to groom was highly variable, and SAHA-treated rats drinking control liquid diet exhibited an increased latency to groom compared with vehicle-treated controls, indicating that SAHA delays the initiation of behavior in the control rats. Nonetheless, SAHA-treated rats drinking control diet did not significantly differ in total grooming time when compared to vehicle-treated controls.

We next confirmed depression-like behavior during withdrawal from chronic ethanol drinking using the sucrose preference test. Ethanol-withdrawn rats had decreased sucrose preference compared with control rats, and treatment with SAHA increased preference for sucrose to control levels (Fig. 1A). A two-way ANOVA indicated a significant ethanol withdrawal × SAHA interaction in sucrose preference [F(1,57) = 11.18, p = 0.0015], but no main effects of ethanol withdrawal or SAHA treatment [withdrawal: F(1,57) = 0.73, p = 0.398; SAHA: F(1,57) = 1.16, p = 0.287]. Post hoc multiple comparisons tests showed a significant difference between vehicle-treated control vs. ethanol-withdrawn rats (p = 0.028) and vehicle-treated, ethanol-withdrawn rats vs. SAHA-treated, ethanol-withdrawn rats (p = 0.018). These results indicate that depression-like behavior is observed in rats during withdrawal from chronic ethanol drinking and that acute treatment with SAHA during withdrawal attenuates the depression phenotype.

**Increased Hdac2 mRNA expression in the hippocampus during chronic ethanol drinking and withdrawal**

We next examined the expression of Hdac2 transcript in the hippocampus during withdrawal. Hdac2 mRNA expression was higher in the CA1, CA3, and DG regions of the hippocampus during ethanol drinking and withdrawal by *in situ* RT-PCR (Fig. 2). There were significant treatment effects by one-way ANOVA in all three regions [CA1, F(2,17) = 6.38, p = 0.0086; CA3, F(2,14) = 11.41, p = 0.0012; DG, F(2,12) = 16.14, p = 0.0004]. Post hoc multiple comparisons tests showed that Hdac2 expression increased in the CA1 (p = 0.0072), CA3 (p = 0.0012), and DG (p = 0.0052) during withdrawal when compared with the control group. Moreover, Hdac2 expression increased in the CA3 (p = 0.012) and DG (p = 0.0052) regions of the ethanol-drinking group (no withdrawal) compared with the control group. These results indicate that chronic ethanol drinking and withdrawal result in increased expression of Hdac2 mRNA in the hippocampus.

**Increased HDAC2 protein in the hippocampus during withdrawal from chronic ethanol drinking is normalized by SAHA treatment**

To determine whether the increase in Hdac2 mRNA in the hippocampus during ethanol withdrawal results in an increase in HDAC2 protein, we next measured protein levels of HDAC2 in the hippocampus using the gold immunolabeling procedure. HDAC2 protein levels were significantly increased in the CA1, CA3, and DG regions of the hippocampus during withdrawal (Fig. 3). Notably, HDAC2 protein returned to control levels when rats were treated with SAHA during withdrawal (Fig. 3). One-way ANOVAs revealed significant effects of treatment in all three regions of the hippocampus [CA1, F(4,20) = 19.29, p < 0.0001; CA3, F(4,20) = 16.65, p < 0.0001; DG: F(4,20) = 38.10, p < 0.0001], with post hoc multiple comparisons tests showing a significant increase in HDAC2 expression in rats treated with vehicle during withdrawal compared with vehicle-treated control diet rats (p < 0.0001 for CA1, CA3, and DG), and a significant decrease in HDAC2 expression in rats treated with SAHA during withdrawal when compared with the withdrawal-vehicle group (p < 0.0001 for CA1, CA3, and DG). These results indicate that, in addition to increased *Hdac2* transcript in the hippocampus during ethanol withdrawal, there is an associated increase in HDAC2 protein, which is normalized by SAHA treatment.

**Decreased H3K9ac in the hippocampus during withdrawal from chronic ethanol drinking is normalized by SAHA treatment**

Increased HDAC2 expression in the hippocampus during withdrawal from chronic ethanol drinking is predicted to cause a corresponding decrease in histone acetylation, while inhibition of HDACs with SAHA would increase histone acetylation. We measured total H3K9ac in the hippocampus during ethanol withdrawal using gold immunolabeling. There was a significant reduction in H3K9ac immunogold particles in the CA3 region during ethanol withdrawal that returned to control levels after SAHA treatment (Fig. 4). A one-way ANOVA showed a significant treatment effect [F(4,20) = 18.47, p < 0.0001], with post hoc comparisons demonstrating a significant decrease in H3K9ac in withdrawal-vehicle rats compared with all other groups (p < 0.0001). H3K9ac was not altered in the DG or CA1 regions during withdrawal [one-way ANOVA, DG: F(4,20) = 0.23, p = 0.92; CA1: F(4,20) = 0.128, p = 0.97]. Together, these results indicate that hippocampal CA3 H3K9ac levels are decreased during withdrawal from chronic ethanol exposure and that normal levels can be restored by treatment with the HDAC inhibitor SAHA.

**Discussion**

Withdrawal from alcohol drinking causes negative mood states that contribute to relapse, including anxiety and depression. Several studies in rats and mice have shown that depression-like
behavior is induced during ethanol withdrawal (Antón et al., 2017; Getachew et al., 2017; Kang et al., 2018; Li et al., 2017; Pang et al., 2013; Roni & Rahman, 2017; Walker et al., 2010; Yawalkar et al., 2018). In this study, we confirmed that rats exhibit depression-like behavior during withdrawal from an ethanol liquid diet using two behavioral measures and demonstrate that systemic treatment with the HDAC inhibitor SAHA during withdrawal alleviates depression-like behavior. In addition, we showed that ethanol withdrawal increases HDAC2 expression, with an associated decrease in H3K9ac in the hippocampus, alterations that are normalized by acute treatment with SAHA during ethanol withdrawal. This effect was apparent 2 h following treatment with SAHA, which is consistent with previous studies demonstrating that acute treatment with the HDAC inhibitor TSA can reduce anxiety-like behavior, increase Npy, Bdnf, and Arc gene expression, and increase dendritic spine density in the central and medial nuclei of the amygdala in a similar time frame (Pandey et al., 2008; You et al., 2014). In addition, 2 h after treatment with SAHA, H3K9ac and sensitivity to GABA in the VTA are increased (You et al., 2018). Collectively, these data indicate that SAHA can act fairly rapidly to alter both gene expression and behavior. Together, our results demonstrate that treatment with SAHA might be an effective strategy to reduce depression during alcohol withdrawal and identify an epigenetic change in the hippocampus as a potential molecular contributor to this condition.

Several behavioral tests have been used to measure depression-like behavior in rats and mice. These include the forced swim test (FST), sucrose preference test, learned helplessness test, and splash test (Akil et al., 2018). Studies of depression-like behavior during ethanol withdrawal in rats have employed the FST and found increased immobility time in animals during withdrawal (Antón et al., 2017; Getachew et al., 2017; Kang et al., 2018; Li et al., 2017; Walker et al., 2010; Yawalkar et al., 2018). In this study, we used the splash test because it is a straightforward measure of depression that takes advantage of the natural propensity of the animal to groom, with less grooming time indicating decreased motivation for self-care. This characteristic is similar in humans diagnosed with depression. In addition, the splash test does not require any prior habituation session, which is needed when performing the FST with rats (Slattery & Cryan, 2012), thus allowing...
the investigation of depression-like behavior after 24 h of withdrawal without prior training during the intoxication period. Decreased grooming in the splash test is observed after chronic unpredictable mild stress, a well-validated model of depression (Hu et al., 2017; Isingrini et al., 2010; Surget et al., 2008; Yalcin et al., 2008). The splash test has also been pharmacologically validated using fluoxetine, with fluoxetine treatment increasing grooming time (Marrocco et al., 2014; Masrour et al., 2018; Santarelli et al., 2003; Surget et al., 2008; Yalcin et al., 2008). To our knowledge, this is the first time the splash test has been used to measure depression-like behavior during ethanol withdrawal. We propose that it is an easily employed measure of ethanol withdrawal-induced depression that does not cause undue stress to the animal. Importantly, our findings in the splash test were confirmed using another simple test, the sucrose preference test, which verified that ethanol withdrawal-induced depression-like behavior is ameliorated by SAHA treatment.

Our finding that treatment with SAHA during withdrawal alleviates depression-like behavior is consistent with the effect of SAHA in reducing depression in stress-induced models of depression. Systemic treatment of mice with SAHA during chronic mild unpredictable stress sessions reduced depression- and anxiety-like behavior as measured using the social interaction test, sucrose preference test, novelty-suppressed feeding, and FST (Uchida et al., 2011). Microinjection of SAHA into the nucleus accumbens reversed stress-induced social aversion and decreased immobility time in the FST (Covington et al., 2009). In a different model of depression involving repeated injections of corticosterone in mice, SAHA corrected several measures of anxiety and depression, including grooming time in the splash test, and normalized hypothalamic–pituitary–adrenal axis reactivity and inflammatory-related gene expression in the hippocampus (Kv et al., 2018). SAHA was also able to decrease depression-like behavior exhibited by mice with a knockout of the CREB-regulated transcription coactivator-1 (Crtc1) gene when mice were tested in a repeated open-space forced swim procedure, but not in the novelty-suppressed feeding test (Meylan, Halfon, Magistretti, & Cardinaux, 2016). Overall, SAHA appears to be an effective treatment for anxiety and depression in multiple animal models of these psychopathological conditions.

Previous work found that treatment with the HDAC inhibitor TSA alleviated ethanol withdrawal-induced anxiety, as measured using the light-dark box and elevated plus maze (Pandey et al., 2008; You et al., 2014). Our results with the splash and sucrose preference tests add another dimension to the negative affective state observed during ethanol withdrawal using the same treatment paradigm, and suggest that rats undergoing withdrawal not only demonstrate anxiety-like behaviors but also depression-like behaviors as indicated in the current study. The effect of SAHA on depression-like behavior during withdrawal is consistent with the effect of TSA on anxiety-like behavior. Both SAHA and TSA are pan-HDAC inhibitors, so the specific HDAC target of these compounds in decreasing anxiety and depression is not known. We demonstrate here that HDAC2 transcript and protein expression are higher in the hippocampus during ethanol withdrawal. Previous studies have found that the innately anxious and high alcohol-preferring P rats have increased HDAC2 in the amygdala, and infusion of an HDAC2 siRNA into the amygdala or treatment with TSA decreased anxiety-like behavior and ameliorated depression-like behavior is consistent with the effect of TSA on anxiety-like behavior. Both SAHA and TSA are pan-HDAC inhibitors, so the specific HDAC target of these compounds in decreasing anxiety and depression is not known. We demonstrate here that HDAC2 transcript and protein expression are higher in the hippocampus during ethanol withdrawal. Previous studies have found that the innately anxious and high alcohol-preferring P rats have increased HDAC2 in the amygdala, and infusion of an HDAC2 siRNA into the amygdala or treatment with TSA decreased anxiety-like behavior and ameliorated depression-like behavior.
like and alcohol drinking behaviors (Moonat, Sakhrkar, Zhang, Tang, & Pandey, 2013; Sakhrkar et al., 2014). HDAC2 expression in the amygdala is also observed after binge alcohol exposure in rats (Lopez-Moreno et al., 2015) and in adulthood after adolescent alcohol exposure (Pandey, Sakhrkar, Tang, & Zhang, 2015). Interestingly, treatment with TSA was able to attenuate adolescent alcohol exposure-induced anxiety-like and alcohol drinking behaviors in adulthood (Pandey et al., 2015). These results implicate HDAC2 as a putative target of SAHA involved in depression-like behaviors in adulthood (Pandey et al., 2015). This putative target gene modulated by HDAC2 might be involved in depression during alcohol withdrawal is Bdnf.

Numerous evidence supports the hypothesis that low levels of BDNF in the hippocampus are associated with depression (Bjorkholm & Monteggia, 2016) and that Bdnf expression is regulated by HDAC9ac through the histone acetyltransferase CREB binding protein (CBP) (Hing, Sathyaputri, & Potash, 2018) and HDAC2 (Guang et al., 2009). Decreased BDNF in the hippocampus is observed during alcohol withdrawal in rats, which is normalized by antidepressant treatment (Hauser, Getachew, Taylor, & Tizabi, 2011). In addition, adolescent rats exposed to a binge ethanol exposure protocol had decreased BDNF in the hippocampus and exhibited depression-like behavior during withdrawal, which was ameliorated by intra-hippocampal infusion of an agonist to the BDNF receptor TrkB (Briones & Woods, 2013). However, other genes involved in depression might also be regulated by histone acetylation during ethanol withdrawal.

Increased inflammation-related
genes such as cytokines and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling molecules are also observed in the hippocampus during ethanol withdrawal (Doremus-Fitzwater et al., 2014; Gano, Doremus-Fitzwater, & Deak, 2016; He & Crews, 2008; Kane et al., 2014; Pascual, Balino, Aragón, & Guerri, 2015), and HDAC inhibitors reduce expression of neuro-immune genes in glial cells in culture and in the brain (Faraco et al., 2009; Kazantsev & Thompson, 2008; Patnala, Arumugam, Gupta, & Dheen, 2017). It is likely that multiple genes expressed in the hippocampus are under epigenetic control and contribute to the negative affective state during alcohol withdrawal. Investigation into these epigenetic mechanisms will no doubt lead to potential new therapeutic targets for the treatment of comorbid AUD and depression.

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Conflicts of interest

No biomedical financial interests or potential conflicts of interest were reported by authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.alcohol.2019.02.005.

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