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Chronic activation of fear engrams induces extinction-like behavior in ethanol-exposed mice

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Abstract

Alcohol withdrawal directly impacts the brain's stress and memory systems, which may underlie individual susceptibility to persistent drug and alcohol-seeking behaviors. Numerous studies demonstrate that forced alcohol abstinence, which may lead to withdrawal, can impair fear-related memory processes in rodents such as extinction learning; however, the underlying neural circuits mediating these impairments remain elusive. Here, we tested an optogenetic strategy aimed at mitigating fear extinction retrieval impairments in male c57BL/6 mice following exposure to alcohol (i.e., ethanol) and forced abstinence. In the first experiment, extensive behavioral extinction training in a fear-conditioned context was impaired in ethanol-exposed mice compared to controls. In the second experiment, neuronal ensembles processing a contextual fear memory in the dorsal hippocampus were tagged and optogenetically reactivated repeatedly in a distinct context in ethanol-exposed and control mice. Chronic activation of these cells resulted in a context-specific, extinction-like reduction in fear responses in both control and ethanol-exposed mice. These findings suggest that while ethanol can impair the retrieval an extinction memory, optogenetic manipulation of a fear engram is sufficient to induce an extinction-like reduction in fear responses.

Keywords

addiction; alcohol; engram; extinction; fear conditioning; hippocampus; optogenetics; withdrawal

1 | INTRODUCTION

More than 50% of people who undergo formal treatment for an alcohol use disorder (AUD) relapse before they can reach a year of sobriety (Connor, Haber, & Hall, 2016). A major factor contributing to the susceptibility of relapse involves the direct, negative

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CONFLICT OF INTERESTS

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impact that withdrawal from alcohol has on stress and memory systems (Koob, 2008; Staples & Mandyam, 2016). Exposure to drug-associated cues, including contextual cues, can trigger memories of past alcohol-drinking experiences, increase levels of stress-related hormones, and potentiate relapse-related behavior (Chaudhri, Sahuque, & Janak, 2008). The hippocampus, a key brain region involved in both stress and memory processes, is critical in the formation of drug-context associations and therefore may be a potential neural target to mitigate stress- and memory-induced relapse behavior in AUDs (Good & Maren, 2019).

Exposure-based therapies—founded upon the behavioral principles of extinction learning (Bouton, 2019; Clem & Schiller, 2016; Good & Maren, 2019)—have been somewhat successful in the clinical treatment of anxiety, stress, and addiction disorders (Kaplan, Heinrichs, & Carey, 2011; Mellentin et al., 2017). In extinction learning, exposure to a conditioned stimulus (context) in the absence of an unconditioned stimulus (shock) leads to a reduction in conditioned behavior (e.g., freezing in the case of fear-conditioning) (Bouton, 2019). Although exposure-based therapies have been used as a treatment option for addiction, their moderate success is likely related to the fact that preclinical studies demonstrate significant impairments in extinction processes in rodent models of alcohol (i.e., ethanol) use and withdrawal. For example, ethanol dependence can induce increased resistance to extinction training, where more sessions to reach an extinction criterion are required following intermittent ethanol exposure in adult (Gass, McGonigal, & Chandler, 2017) and adolescent rats (Gass et al., 2014). Furthermore, rodent models also demonstrate that ethanol-related cues, as well as stress, can induce reinstatement and context-dependent renewal of ethanol-associated lever pressing following extinction (Burattini, Gill, Aicardi, & Janak, 2006; Chaudhri et al., 2008; Keistler et al., 2017). These data suggest that aspects of ethanol withdrawal can promote ethanol-seeking behavior potentially through mechanisms of enhanced stress (Koob, 2008) where disruption of extinction occurs, thereby potentially hampering clinical attempts to utilize extinction processes to treat AUDs.

In rodents withdrawn from ethanol, preclinical studies also show impairments in memory processes unrelated to the ethanol itself (e.g., fear learning). These deficits include impaired extinction learning, heightened fear responses, stress-induced reinstatement, and increased fear generalization (Bertotto, Bustos, Molina, & Martijena, 2006; Broadwater & Spear, 2013; Holmes et al., 2012; Scarlata et al., 2019). Further, fear memories formed prior to ethanol exposure and withdrawal also fail to extinguish (Quinones-Laracuente, Hernandez-Rodriguez, Bravo-Rivera, Melendez, & Quirk, 2015), suggesting that withdrawal from ethanol augments the retrieval of fear memories (Scarlata et al., 2019). Additionally, as withdrawal from ethanol induces changes in the neuroendocrine stress response system (Becker, 2012), it is likely that stress-induced changes at cortical, amygdala, and hippocampal structures disrupt general mechanisms of extinction learning (Maren & Holmes, 2016). If extinction impairments in ethanol-withdrawn rodents reflect retrieval deficits related to the extinction memory (e.g., enhanced fear generalization) this may be manifested as increased freezing in a neutral context (Jasnow, Lynch III, Gilman, & Riccio, 2017; McAllister & McAllister, 1963; Perkins Jr. & Weyant, 1958). In this case, targeting retrieval processes may prove effective at mitigating the extinction impairments observed following withdrawal from ethanol. Here, we employ a strategy that drives context-specific

associative learning that may overcome these retrieval errors by targeting the original fear memory (Chen et al., 2019).

Fear memory retrieval is a dynamic process that can be measured at the neuronal, circuit, and behavioral level. Moreover, a behavioral strategy to suppress fear and induce extinction learning involves returning a rodent to the fear-conditioning context in the absence of shock (Good & Maren, 2019). Through repeated extinction sessions, the retrieval of a fear memory in the absence of the unconditioned stimulus leads to an overall reduction in conditioned response (i.e., freezing (Good & Maren, 2019)). An alternative method involves an artificial strategy, which attempts to optogenetically activate a fear engram—the neuronal ensemble active during acquisition which undergoes plasticity, and which reactivation of facilitates retrieval (Chen et al., 2019; Josselyn & Tonegawa, 2020; Liu et al., 2012; Ramirez et al., 2015). Promisingly, optogenetic activation of cells in the dorsal dentate gyrus (dDG) subregion of the hippocampus that were previously active during fear learning is sufficient to activate the neuronal and behavioral expression of memory recall (Liu et al., 2012; Ramirez et al., 2013, 2015). Recently, we demonstrated that repeated (or “chronic”) optical activation of a fear memory in the dDG leads to a context-specific, extinction-like reduction in freezing (Chen et al., 2019). Therefore, we asked if repeated activation of a hippocampal fear engram could mitigate forced abstinence-induced fear extinction deficits, possibly caused by acute withdrawal, in a context-specific manner.

We first utilized a behavioral strategy to evaluate aberrant fear extinction in a mouse model of chronic ethanol exposure and forced abstinence. All subjects were treated in accordance with protocol 17-008 approved by the Institutional Animal Care and Use Committee at Boston University. Adult male mice received intraperitoneal injections of 30% ethanol (vol/vol) in saline (0.9%) at a dose of 2.0 g/kg (EtOH) or saline (Sal) for five consecutive days, followed by a 2-day forced abstinence period (Pina & Cunningham, 2017; Quinones-Laracuente et al., 2015). Twenty-eight male C57Bl6/J mice (Sal = 14, EtOH = 14) were fear conditioned in two distinct contexts, Context A (Ctx A) and Context B (Ctx B), extinguished in Ctx A, and tested in both Ctx A and B as previously described (Chen et al., 2019; Figure 1a).

We observed no group differences in fear acquisition in Ctx A (Figure 1b); however, there was a main effect of trial demonstrating increases in freezing in the latter trials compared to baseline and earlier trials (two-way RM analysis of variance [ANOVA]; $F_{(4,72)} = 54.98$, $p < .0001$). This was also the case in Ctx B (Figure 1c) (two-way RM ANOVA; $F_{(4,72)} = 15.54$, $p < .0001$). Following fear acquisition, mice underwent 10-min extinction sessions in Ctx A (2 sessions per day for 5 days). Across extinction, we observed a gradual decrease in freezing levels (Figure 1d) (two-way RM ANOVA, session) ($F_{(9,234)} = 65.47$, $p < .0001$). Qualitatively, we observed that as extinction sessions progressed, EtOH-exposed mice were freezing to a greater extent than Sal-control mice, specifically during the first extinction session on a given day. To account for time of day as a factor, we analyzed freezing levels during the AM and PM extinction session of EtOH-exposed and Sal-control mice for each day of extinction. This analysis revealed a main effect of day, main effect of time of day, and main effect of treatment (three-way RM ANOVA) (day $F_{(4,130)} = 24.23$, $p < .0001$, time of day $F_{(1,130)} = 139.5$, $p < .0001$, treatment $F_{(1,130)} = 6.612$, $p = .0113$). (Figure

1e) Interestingly, the interaction effect of time of day × treatment revealed a strong, albeit nonsignificant, trend (three-way RM ANOVA, time of day × treatment $F_{(1,130)} = 3.530$, $p = .0625$) (Figure 1e).

Following extinction, mice were tested for contextual fear memory recall in Ctx A (Figure 1f). If extinction impairments in EtOH-exposed mice reflect fear memory extinction retrieval deficits, this would result in an increase in freezing. As such, we expected freezing levels to be higher in EtOH-exposed mice relative to Sal-control mice and therefore ran a one-tailed unpaired *t* test, which we observed to be the case (unpaired *t* test, one-tailed, t(26) = 2.020, $p = .0269$). In contrast, no group differences were found in freezing during the recall test in Ctx B (unpaired *t* test, one-tailed, $t(26) = 0.968$, $p = .1709$) (Figure 1g). Following behavior, cFos + cells in the dDG were quantified as a proxy of activation during recall in Ctx A (Figure 1h,i). We observed no significant difference in this cellular marker of activity during recall (unpaired *t* test, two-tailed, $t_{(5)} = 0.629$, $p = .5567$) (Figure 1h). Together, these data show that EtOH-exposed have an extinction retrieval deficit as evident by a higher degree of context-specific freezing during recall, despite comparable levels of cFos activation in the dDG.

Our previous work showed that chronic stimulation of cells in the dorsal hippocampus active during the formation a fear memory led to an extinction-like, context-specific reduction in fear behavior in mice (Chen et al., 2019). Therefore, we asked if chronic optogenetic activation of a fear engram in the dDG could facilitate extinction learning in EtOH-exposed mice. Forty-four adult male mice were placed on a diet containing doxycycline (40 mg/kg; Dox). Briefly, mice were injected with a virus cocktail ($pAAV_9$-c-Fos-tTA and either $pAAV_9$-TRE-ChR2-eYFP [ChR2] or $pAAV_9$-TRE-eYFP [eYFP]) into the dDG followed by bilateral optic fiber implantation (Figure 2a,b; see Chen et al., 2019; Ramirez et al., 2015).

Following recovery, mice received 5 days of ethanol or saline treatment. As in the previous experiment, mice underwent a two-day forced abstinence period prior to fear conditioning. Mice were taken off-Dox 24 hr prior to fear conditioning in Ctx A, and immediately put back on-Dox after tagging for subsequent fear conditioning in Ctx B. Over the next 5 days, mice were placed into a distinct context (Ctx C) and received repeated light-stimulation (473 nm, 20 Hz) over a 10-min session, twice a day for 5 days, as previously reported (Chen et al., 2019; Ramirez et al., 2015). Finally, mice were tested for contextual fear memory recall in both Ctx A and Ctx B (Figure 2c). Analysis of freezing during optogenetic activation sessions revealed a main effect of session, a main effect of virus, and no main effect of treatment (three-way RM ANOVA) (session $F_{(9,216)} = 44.57$, $p < .0001$, virus $F_{(1,216)} = 38.74$, $p < .0001$) (Figure 2d). Differences in freezing between EtOH-exposed ChR2 and eYFP groups, as well as Sal-control ChR2 and eYFP groups, indicated significant light-induced freezing from reactivation of the initial tagged fear conditioning session (Figure 2d), and equal levels of light-induced freezing were observed in each ChR2 treatment group (unpaired *t* test on difference score, two-tailed, $t_{(18)} = 0.3030$, $p = .7654$; Figure 2e).

We measured freezing in Ctx A and Ctx B for ChR2 EtOH-exposed and Sal-control mice as well as eYFP controls. Overall, we found a main effect of context, a main effect

of virus, and no main effect of treatment. We found a significant context by treatment interaction (three-way RM ANOVA) ($F_{(1,40)} = 4.274$, $p = .0452$). This effect is driven by the observation that across treatment, there is a significantly lower degree of freezing in Ctx A for ChR2 animals (Tukey's HSD, EtOH-ChR2: $p < .001$; Sal-ChR2: $p = .032$), suggesting that chronic optogenetic activation induced a context-specific decrease in freezing. This effect was not observed in eYFP mice. Additionally, there was a significant context x virus interaction (three-way RM ANOVA) ($F_{(1,40)} = 7.721$, $p = .0083$) and this effect was due to the difference in freezing within Ctx A, between the EtOH ChR2 group and the Sal eYFP group (Tukey's HSD, $p < .001$) and between ChR2 and eYFP mice overall (Tukey's HSD, ChR2 vs. eYFP: $p < .001$) (Figure 2f).

Following behavior, cFos + cells in the dDG were quantified as a proxy of activation during recall in Ctx A (Figure 2g,h). We observed no significant difference in cellular activity during recall (unpaired *t* test, two-tailed, $t_{(6)} = 1.6$, $p = .1606$) (Figure 2f). Together, these data demonstrate that chronic activation of a tagged dDG fear memory leads to a context-specific reduction in freezing in both ChR2 EtOH and Sal mice.

We utilized a behavioral and optogenetic approach to respectively evaluate and reduce aberrant fear extinction responses in mice that experienced ethanol exposure and forced abstinence. Although both groups of mice acquired fear to a similar extent to both contexts, EtOH-exposed mice showed heightened freezing during the baseline preshock period during the fear conditioning session in Ctx B. At the end of the fear conditioning session both groups acquired equal levels of freezing, suggestive of a potential ceiling effect that may have occluded underlying effects of ethanol-induced generalization or enhanced fear learning in Ctx B. Future studies that may reduce the intensity of the unconditioned stimulus or include a habituation session to Ctx B prior to fear conditioning could potentially reveal effects of ethanol related to fear generalization.

Our analysis of the 10 extinction sessions did not reveal a significant difference in extinction training or learning between EtOH-exposed and Sal-control animals. However, EtOH-exposed mice exhibited freezing behavior at a qualitatively and noticeably higher level than Sal-control mice, specifically during the morning extinction session on a given day. Interestingly, analysis including time of day as a factor revealed a significant main effect of treatment (Figure 1e). To our knowledge, our extinction paradigm used here is a novel approach to extinguishing fear memories in rodents withdrawn from ethanol, as previous studies that have shown extinction learning deficits in rodents withdrawn from alcohol have used single extinction sessions per 24-hr interval while we utilized two sessions per day in our paradigm (Bertotto et al., 2006; Broadwater & Spear, 2013; Holmes et al., 2012; Quinones-Laracuente et al., 2015). The extinction paradigm used here was selected to emulate the chronic activation paradigm previously shown to successfully reduce freezing responses in mice (Chen et al., 2019), and we speculate that the increased number of extinction sessions per day mitigated the expected extinction-learning impairments. Nonetheless, EtOH-exposed mice exhibited context-specific extinction retrieval deficits (i.e., heightened fear responses during Ctx A recall tests, Figure 1f) relative to Sal-control mice following extinction.

Next, chronic optogenetic activation of tagged dDG cells induced a context-specific, extinction-like reduction of freezing behavior in both Sal-control and EtOH-exposed mice, but not in eYFP controls. Moreover, freezing levels in the tagged context did not differ between ChR2 EtOH-exposed and Sal-control mice. These data lend credence to the idea that artificially reactivating a fear memory over multiple sessions is sufficient to reduce fear memories in an extinction-like manner in both Sal-control and EtOH-exposed mice, and points to dDG-mediated engrams as a key node that, when modulated, may lead to a reduction in maladaptive behavioral responses associated with anxiety and fear-related disorders (Josselyn & Tonegawa, 2020; Ramirez et al., 2015).

We hypothesize that forced abstinence of ethanol may have caused withdrawal-induced changes to stress systems in our mice, which led to a general impairment in extinction retrieval following extinction training. It is possible that the ability of EtOH-exposed mice to attenuate their fear memories was resistant to behavioral extinction, but susceptible to optogenetic perturbations. Extinction-retrieval impairments in the EtOH-exposed mice reported here dovetails with previous reports of ethanol-withdrawal memory impairments, including heightened fear responses, increased context generalization, impaired extinction processes, and stress/cue-induced reinstatement in rodents (Bertotto et al., 2006; Broadwater & Spear, 2013; Holmes et al., 2012; Quinones-Laracuente et al., 2015).

Additionally, we found no difference in cFos + cell quantifications between EtOH-exposed and Sal-control groups in both the normative and optogenetic experiments. Though the sample sizes used here were based on previous literature (Chen et al., 2019; Sun et al., 2020; Zhang, Kim, & Tonegawa, 2020), future studies may increase sample sizes to potentially detect subtle differences across groups. Further, it is possible that an upstream brain region mediates the behavioral impairment observed in normative EtOH-exposed mice; for instance, previous studies regarding fear memory retrieval altered by ethanol have found increased cFos in the prelimbic cortex, paraventricular thalamus, and medial central amygdala, and have found these regions function as a circuit during fear retrieval (Do-Monte, Quiñones-Laracuente, & Quirk, 2015; Quinones-Laracuente et al., 2015). In our experiments, we speculate that by perturbating an upstream hippocampal node implicated in processing the contextual components of a fear memory engram, we optogenetically engaged independent circuitry capable of attenuating fear responses and thus artificially facilitated extinction-like behaviors in both EtOH-exposed and Sal-control mice. In line with this view, and despite our negative cFos data in Figure 2, we propose that our chronic stimulation protocol bypassed numerous brain-wide systems previously implicated in mediating addiction-related behaviors, such as the insular cortex, which governs interoceptive feelings of drug craving and withdrawal (Naqvi & Bechara, 2009). Measuring immediate early genes in the insular cortex may reveal differential activity levels between EtOH-exposed and Sal-control mice, which potentially underlie the impaired ability of EtOH-exposed mice to mitigate fear memories.

Moreover, recent studies have shown that discrete populations of cells contributing to a memory engram have distinct cellular activity, synaptic properties, and behavioral results dependent on the immediate early gene used to identify a given cellular ensemble (Sun et al., 2020). Specifically, cFos-mediated engrams have been implicated in promoting

memory generalization, such that acute chemogenetic activation of a contextual fear memory led to reduced discrimination between distinct contexts (Sun et al., 2020). In parallel, chronic activation of a fear memory in a neutral context may have promoted generalization between the neutral and conditioned context, as evident by decreased freezing in the conditioned context. Previously reported pharmacological and optogenetic approaches that mitigate stress- and addiction-related behavioral states have demonstrated enduring changes in behavior following sustained, but not acute, activation of neural systems (Ramirez et al., 2015); likewise, we posit that our chronic activation protocol may enduringly reprogram and/or modify existing engrams and circuits sufficient to alleviate addiction-related behavioral states.

Alternatively, chronic optogenetic activation may cause an artificial extinction-like engram to emerge in the DG. Previous studies have demonstrated that extinction recruits a new population of DG cells that compete with the original fear engram to drive each corresponding behavior (Chen et al., 2019; Khalaf et al., 2018; Lacagnina et al., 2019). In our recent study, we observed that chronic stimulation of DG-mediated engrams led to a reduction of cFos activity in the originally tagged population of cells while the DG nonetheless maintained a similar level of overall cFos expression levels in non-tagged populations, suggesting too that a distinct engram had simultaneously emerged—this artificial engram may function to suppress freezing behavior elicited by the original fear engram (Chen et al., 2019). Likewise, we believe that consistent with recent work, the dentate gyrus may contain both fear and extinction engrams that may mutually modulate each other (e.g., “fear cells” may be under local inhibition from “extinction cells”) (Lacagnina et al., 2019). We posit that this phenomenon is unaffected by the underlying mechanisms of ethanol exposure and forced abstinence which led to the extinction-retrieval impairment observed in normative extinction learning, and we would expect to see a similar decrease in cFos expression in the tagged fear memory engram following chronic activation in both Sal-control and EtOH-exposed mice.

Taken together, the emergence of novel strategies sufficient to mitigate fear responses holds promising value in better understanding the underlying mechanisms of learning and memory. In particular, modulating addiction-related engrams permits a brain-wide cataloguing of the maladaptive structural and functional changes while pointing to key cellular mechanisms that may be sites of future intervention (Whitaker & Hope, 2018). By monitoring and manipulating the cellular, circuit, and systems-wide changes that occur when a brain transitions into a state of drug or alcohol dependence, we believe it may be possible to artificially and enduringly restore healthy neuronal functioning and corresponding behavioral outputs.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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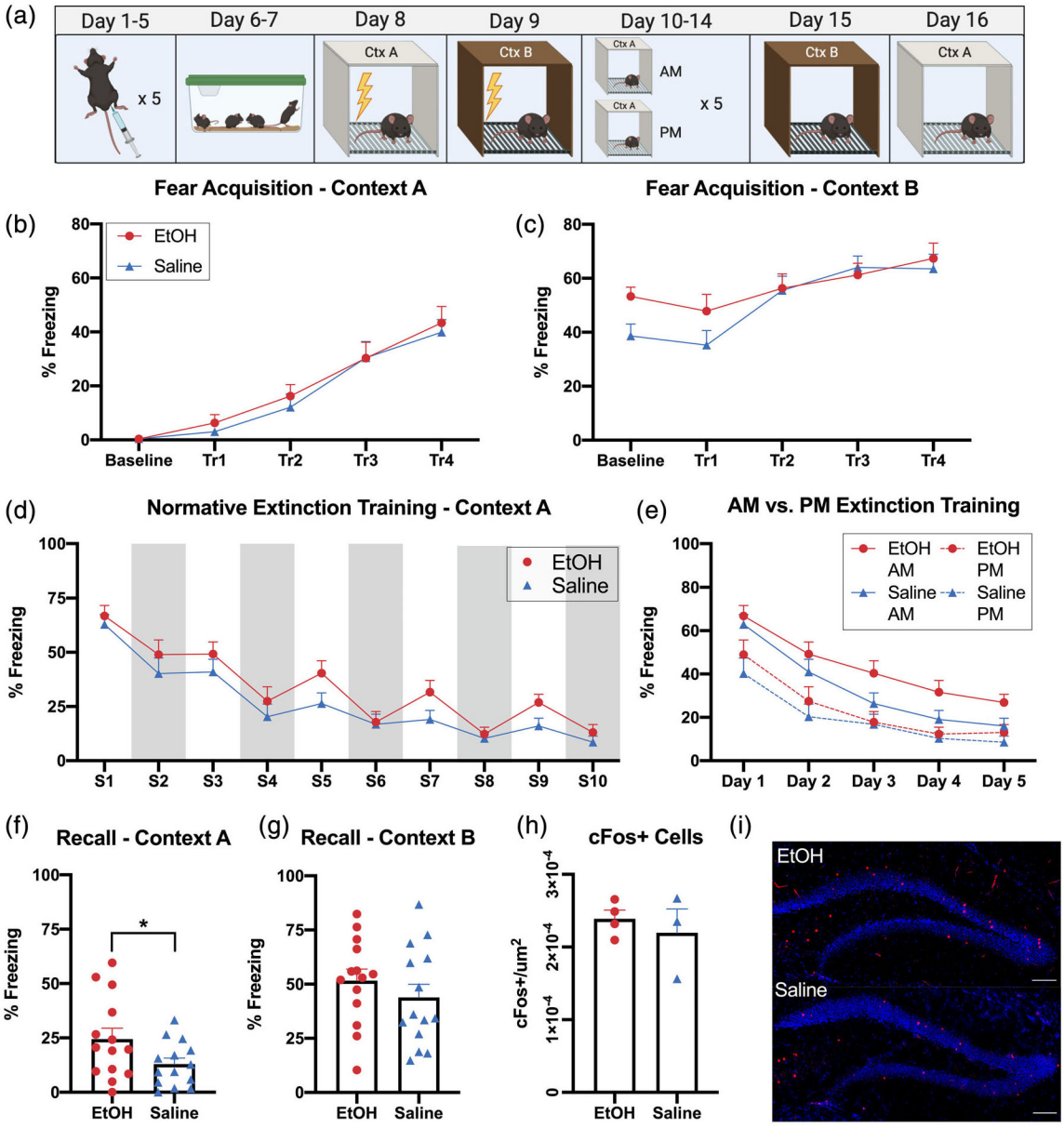

FIGURE 1.

Ethanol exposure and forced abstinence impairs normative extinction learning. (a) Behavioral design for ethanol (EtOH) or saline exposure, forced abstinence, fear conditioning, extinction, and recall. (b) Fear acquisition of EtOH-exposed and Sal-control mice in Ctx A. No differences between groups (two-way RM analysis of variance [ANOVA], trial) ($F_{(4,72)} = 54.98$, $p < .0001$). (c) Fear acquisition of EtOH-exposed and Sal-control mice in Ctx B. No differences between groups (two-way RM analysis of variance [ANOVA], trial) ($F_{(4,72)} = 15.54$, $p < .0001$). (d) Normative extinction training of EtOH-exposed and Sal-control mice (two-way RM ANOVA, session) ($F_{(9,234)} = 65.47$, $p < .0001$). (e) Comparison of freezing during AM and PM extinction training reveals a group (treatment) differenced, likely based on time of day (three-way RM ANOVA) (day $F_{(4,130)} = 24.23$, $p < .0001$, time of day $F_{(1,130)} = 139.5$ $p < .0001$, treatment $F_{(1,130)} =$

6.612 $p = .0113$, time of day × treatment $F_{(1,130)} = 3.530$ $p = .0625$). (f) Recall in Context A following extinction reveals increased freezing in EtOH-withdrawn mice, indicative of extinction-retrieval deficit (unpaired *t* test, one-tailed, $t_{(26)} = 2.020$, $p = .0269$). (g) In contrast, no group differences were found in freezing during the recall test in Context B (unpaired *t* test, one-tailed, $t_{(26)} = 0.968$, $p = .1709$). (h) Quantification of dorsal dentate gyrus (dDG) cFos + cells during recall in Context A reveals no significant difference in activation (unpaired *t* test, two-tailed, $t_{(5)} = 0.629$, $p = .5567$). (i) Representative histology for cFos + quantification. Scale bar = 100 μm

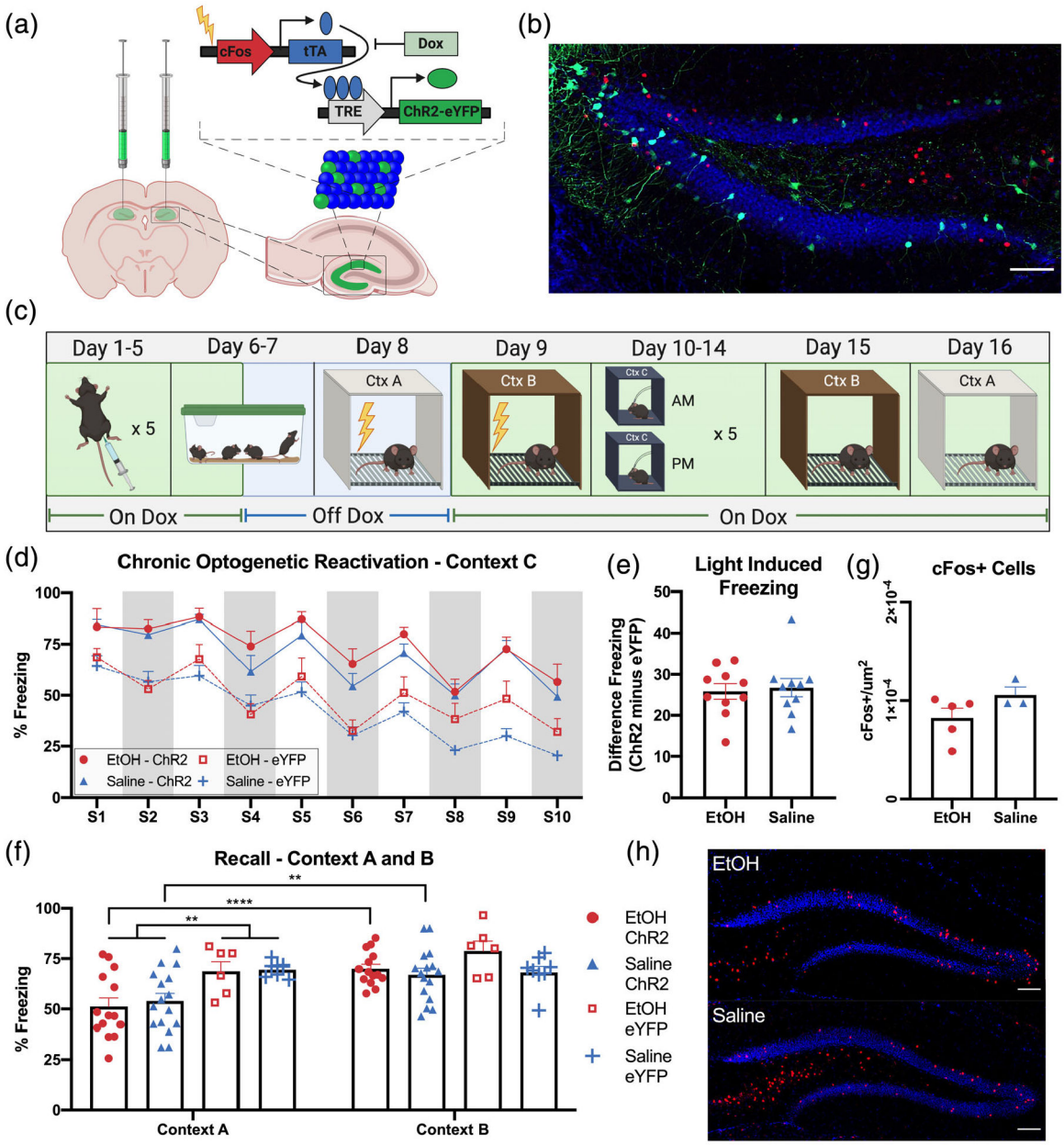

FIGURE 2.

Chronic optogenetic activation reduces freezing in ethanol-exposed mice. (a) Schematic of viral strategy. A viral cocktail of AAV9-c-Fos-tTA and either AAV9-TRE-ChR2-eYFP or AAV9-TRE-eYFP was infused into the dorsal dentate gyrus (dDG) for activity-dependent transcription of ChR2 or eYFP. (b) Representative histology for activity-dependent tagging of contextual fear engrams. Scale bar = 100 μm. (c) Behavioral design for ethanol (EtOH) or saline exposure, forced abstinence, fear conditioning, chronic optogenetic activation, and recall. (d) Freezing levels during chronic activation of a contextual fear engram in neutral Context C. While there is an overall decrease in freezing across all sessions, we found no significant group differences based on treatment for both ChR2 and eYFP groups (three-way RM analysis of variance [ANOVA]) (session $F_{(9,216)} = 44.57$, $p < .0001$, virus

$F_{(1,216)} = 38.74$, $p < .0001$). (e) Difference score for light-induced freezing. For each optogenetic activation session, the difference in freezing between ChR2 and eYFP groups for both EtOH and Sal treatments were calculated. Average difference in freezing is equal for both treatment groups (unpaired *t* test on difference score, two-tailed, $t_{(18)} = 0.3030$, $p = .7654$). (f) Recall in Context A (Ctx A) reveals a significant decrease in freezing levels of ChR2 EtOH and saline mice relative to eYFP control mice. Significant context by treatment interaction (three-way RM ANOVA) ($F_{(1,40)} = 4.274$, $p = .0452$) (Tukey's HSD, EtOH-ChR2: $p < .001$; Sal-ChR2: $p = .032$). Additionally, this decrease in freezing was context specific, with significantly lower freezing levels of EtOH-ChR2 and Sal-ChR2 mice in Ctx A relative to Ctx B. Significant context × virus interaction (three-way RM ANOVA) ($F_{(1,40)} = 7.721$, $p = .0083$) (Tukey's HSD: Ctx A EtOH-ChR2 vs. Sal-ChR2 $p < .001$) suggesting that chronic optogenetic activation induced a context-specific decrease in freezing. (g) Quantification of dDG cFos + cells during recall in Ctx A reveals no significant difference in activation (unpaired *t* test, two-tailed, $t_{(6)} = 1.6$, $p = .1606$). (h) Representative histology for cFos + quantification. Scale bar = 100 μm