HHS Public Access

Author manuscript

Biol Psychiatry. Author manuscript; available in PMC 2024 August 01.

Published in final edited form as:

Biol Psychiatry. 2023 August 01; 94(3): 215–225. doi:10.1016/j.biopsych.2022.12.003.

Oxytocin Reduces Sensitized Stress-Induced Alcohol Relapse in a Model of Post-Traumatic Stress Disorder and Alcohol Use Disorder Comorbidity

Howard C. Becker[a,b,c], **Marcelo F. Lopez**[a], **Courtney E. King**[a], **William C. Griffin**[a]

[a]Charleston Alcohol Research Center, Department of Psychiatry and Behavioral Sciences, Medical University of South Carolina

[b]Department of Neuroscience, Medical University of South Carolina

[c]RHJ Department of Veterans Affairs Medical Center

Abstract

Background: There is high comorbidity of post-traumatic stress disorder (PTSD) and alcohol use disorder (AUD) with few effective treatment options. Animal models of PTSD have shown increases in alcohol drinking but effects of stress history on subsequent vulnerability to alcohol relapse have not been examined. Here we present a mouse model of PTSD involving chronic multimodal stress exposure that results in long-lasting sensitization to stress-induced alcohol relapse, and this sensitized stress response is blocked by oxytocin (OT) administration.

Methods: Male and female mice trained to self-administer alcohol were exposed to predator odor (TMT) + yohimbine over five consecutive days or left undisturbed. After re-establishing stable alcohol responding/intake, mice were tested under extinction conditions and then all mice were exposed to TMT or context cues previously associated with TMT prior to a reinstatement test session. Separate studies examined messenger RNA expression of *Oxt* and *Oxtr* in hypothalamus following chronic stress exposure. A final study examined the effects of systemic administration of OT on stress-induced alcohol relapse in mice with and without a history of chronic stress experience.

Results: Chronic stress exposure produced long-lasting sensitization to subsequent stress-induced alcohol relapse that also generalized to stress-related context cues, transcriptional changes in hypothalamic OT system, and OT injected prior to the reinstatement test session completely blocked the sensitized stress-induced alcohol relapse effect.

Corresponding Author: Howard C. Becker, Ph.D., Charleston Alcohol Research Center, Departments of Psychiatry and Neuroscience, Medical University of South Carolina, RHJ Department of Veterans Affairs Medical Center, Charleston, SC 29425, Tel: 843-792-7723, beckerh@musc.edu.

DISCLOSURES

The authors report no biomedical financial interests or potential conflicts of interest.

Conclusions: Collectively, these results provide support for the therapeutic potential of OT, along with highlighting the value of utilizing this model in evaluating other pharmacological interventions for treatment of PTSD-AUD comorbidity.

Keywords

Stress; Alcohol relapse; Oxytocin; Post-traumatic stress disorder; Alcohol use disorder; Comorbidity

INTRODUCTION

A substantial number of individuals suffer with co-occurring stress-related disorders, such as post-traumatic stress disorder (PTSD) and alcohol use disorder (AUD) (1–5). While the prevalence of PTSD and AUD comorbidity is relatively high in the general population, it is especially high in some at-risk groups (military personnel and Veterans) (6–8). Further, co-occurrence of PTSD and AUD is associated with more severe clinical symptoms compared to either disorder alone (9–11). Despite the high prevalence and significant health care burden, there are few treatments that effectively address this clinical problem of PTSD-AUD comorbidity (4,12). Use of animal models is critical for advancing our understanding of underlying mechanisms and providing platforms for evaluating potential new and novel treatment interventions.

Several animal models with varying degrees of face, construct, and predictive validity have been developed to examine the development of PTSD and evaluate possible targets for pharmacological interventions (1,13–17). Many of these models involving various stress exposure paradigms have proven to capture key features of PTSD and several studies have demonstrated increased alcohol self-administration following stress exposure (18–25). However, rarely have these models been employed to examine how such stress (‘traumatic’) experience may influence subsequent stress-related alcohol relapse.

Stress is known to play an important role in triggering alcohol relapse as well as serving as a significant factor in contributing to heavy drinking. There is significant overlap in neurobiological mechanisms and brain circuits underlying stress and alcohol effects (15,26,27), and several stress-related neuropeptide systems have been implicated as potential targets for treating PTSD-AUD comorbidity (28–31). Among these, the neurohormone oxytocin (OT) has emerged as a promising treatment. OT is predominantly synthesized in the paraventricular and supraoptic nuclei of the hypothalamus and released by the posterior pituitary into general circulation. Hypothalamic OT neurons also project to numerous cortical, limbic, and basal ganglia structures where it mediates an array of behavioral effects via interaction with G(q)-coupled OT receptors (32–34). For example, OT is released in response to different stress stimuli, and it can ameliorate symptoms of stress-related disorders such as depression and anxiety (35–37). Preclinical and clinical studies have shown OT to have promise in treating PTSD symptoms (38–45).

OT also has been implicated in alcohol and substance use disorders (46–50). For example, clinical studies have indicated that intranasal OT treatment reduces alcohol withdrawal symptoms (51), craving (52), and brain activation to alcohol-related cues (53,54), although

there are some negative reports (55,56). Evidence from preclinical studies has demonstrated that activation of endogenous hypothalamic OT release or administration of exogenous OT reduces alcohol consumption in several rodent models (57–65). Thus, given the stress-buffering effects of OT and its ability to reduce alcohol drinking, it seems reasonable to suspect that OT may be especially effective in a PTSD model that is linked to alcohol relapse.

Here we describe a novel mouse model that captures many of the key features of PTSD and its impact on alcohol relapse susceptibility. Additionally, we demonstrate the chronic mulitmodal stress model produces long-lasting transcriptional changes in the oxytocin system and that systemic administration of OT blocks sensitized stress-induced alcohol relapse-like behavior in mice with a history of chronic stress experience. Collectively, these results provide additional support for the therapeutic potential of OT, along with highlighting the value of utilizing this model in evaluating other pharmacological interventions for treatment of PTSD-AUD comorbidity.

MATERIALS and METHODS

Subjects

Adult male and female C57BL/6J mice purchased from Jackson Laboratories (Bar Harbor, ME) were individually housed with free access to food and water throughout all phases of the experiments. Mice were housed in a temperature and humidity-controlled animal facility under a reversed 12-hr light/dark cycle (lights on 1800 hr). All procedures were approved by the MUSC Institutional Animal Care and Use Committee and followed the NIH Guide for the Care and Use of Laboratory Animals.

General Study Procedures

To examine the effects of chronic stress exposure on later stress-induced alcohol relapse-like behavior, male and female mice were first trained to self-administer alcohol using standard operant conditioning procedures (details below). Once stable alcohol responding/intake was established, half the mice were exposed to a compound (predator odor + yohimbine) stressor for 5 consecutive days (see below) while the remaining mice served as no-stress controls. After re-establishing stable alcohol responding/intake, mice were tested under extinction conditions for 14 days and then all mice were exposed to the predator odor alone for reinstatement testing. In separate studies, mice were used to examine the stress response (plasma corticosterone levels) and behavioral reactivity (defecation) following the chronic stress procedure.

After establishing a sensitized stress-induced alcohol relapse-like response in mice with a history of chronic stress exposure, follow-up studies were conducted to test the durability and generalizability of this effect. A separate cohort of mice was treated as above, except that following the reinstatement test (~30 days post-stress exposure), mice resumed alcohol self-administration and after a second extinction test period they received a second reinstatement test session (~60 days post-stress exposure). To test the generalizability of this effect, separate groups of male and female mice were similarly trained except the

multimodal stress exposure was presented in a unique context (mouse cage with stripped walls and wire floor). No-stress mice were left undisturbed. After re-establishing stable baseline alcohol responding/intake and then extinction testing, all mice were exposed to the unique context prior to reinstatement testing.

To determine whether the chronic stress exposure alters oxytocin expression in the hypothalamus (mainly paraventricular nucleus), male and female mice were sacrificed 48-hr or 60-days following the 5-day stress exposure regimen and oxytocin (*Oxt)* and oxytocin receptor (*Oxtr)* mRNA levels were measured by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) assays (see below). In a final study, the effects of systemic administration of OT on the sensitized stress-induced alcohol relapse-like response was determined. Separate groups of mice received intraperitoneal (ip.) injection of OT (1 mg/kg) or vehicle (saline) 15-min prior to the 15-min predator odor exposure during reinstatement testing.

Alcohol Self-Administration, Extinction, and Reinstatement Testing Procedures

Mice were trained to orally self-administer alcohol (12% v/v) under a fixed ratio-4 schedule during 20-min daily sessions using procedures previously published (58) and detailed in Supplemental Materials. Operant conditioning sessions were suspended during chronic (5-day) stress exposure and resumed the following week. During extinction testing, lever responding did not result in delivery of alcohol reinforcement or presentation of cues previously associated with alcohol delivery. Reinstatement testing following predator odor exposure was conducted under extinction conditions.

Chronic Stress Exposure

Mice received yohimbine (2 mg/kg) 15-min prior to exposure to the predator odor TMT (2,5-dihydro-2,4,5-trimethylthiazoline; BioSRq, LLC) (0.03 ml; 1% v/v) for 15-min/day over 5 consecutive days. CTL mice received saline injections and then placed in control chambers. For reinstatement testing, all mice were exposed to TMT exposure alone (15-min) just prior to the session. See Supplemental Materials for details.

Plasma Corticosterone and Hypothalamic Oxt and Oxtr mRNA Measurements

Blood samples collected following chronic stress exposure were used for plasma corticosterone measurement by ELISA (see Supplemental Materials for details). Collection of hypothalamic samples, RNA extraction, and TaqMan qRT-PCR assays were performed as previously described (66,67). Details of qRT-PCR primers and assay parameters are provided in Supplemental Materials.

Statistical Analyses

Active lever responses during baseline alcohol self-administration, extinction testing, and reinstatement testing were analyzed by ANOVA using Statistica V13 (TIBCO Software, Inc.), with Group (Stress vs. CTL) and Sex as main factors, and Test (Extinction vs. Reinstatement) as a repeated measure. Plasma corticosterone levels were analyzed by ANOVA, with History (alcohol-naïve vs. alcohol self-administration), Group (Stress vs. CTL) and Sex as between-subject factors and Day (Day-1, Day-5) as a repeated measure.

In separate cohorts of alcohol-naïve mice, Plasma corticosterone levels, defecations, and *Oxt/Oxtr* mRNA expression were analyzed with Group and Sex as main factors. Post-hoc comparisons were performed when appropriate (Newman-Keuls) and significance level for all analyses was set at $p < 0.05$.

RESULTS

A History of Chronic Stress Exposure Sensitizes Later Stress-Induced Alcohol Relapse-Like Behavior in Mice

Male (N= 10–11/group) and female (N= 12/group) mice were first trained to self-administer alcohol and then after extinction responding stabilized, stress (TMT)-induced alcohol relapse-like behavior was evaluated in mice with or without a history of chronic stress exposure (Figure 1A). Baseline responding both prior to and following chronic predator odor exposure did not differ between groups (Stress vs. CTL) or sex (Group × Sex × Time interaction: [F(1,41)= 2.06, p= 0.159]) (Figures 1B and 1C and Figures S1A and S1B). Despite similar responding on the active lever during the final post-Stress/CTL baseline session for males and females, alcohol intake (g/kg) [F(1,32)= 9.31, p= 0.005] and blood alcohol concentrations (BAC) determined immediately following the session [F(1,32)= 5.17, p= 0.030] were significantly higher in females compared to males (Figure S2A and S2B) and, overall, there was a strong correlation between amount consumed and resultant BAC [R^2= 0.62; [F(1,32)= 51.98, p< 0.001] (Figure S2C).

Following extinction testing, predator odor exposure significantly increased alcohol-seeking behavior (active lever responding). This was supported by a main effect of Test [F(1,41)= 78.33, p< 0.001]. Additionally, this effect was significantly greater in male (Figure 1B and C) and female (Figure 1D and E) mice that had prior chronic stress exposure (Group × Test interaction: [F(1,41)= 14.12, p= 0.001]). The magnitude of this enhanced stress-induced reinstatement effect did not differ between males and females (Group × Sex × Test interaction: [F(1,41)< 1.0]). These data indicate that a history of robust stress experience sensitizes both male and female mice to later stress-induced relapse-like behavior.

Separate studies were conducted to validate that our chronic stress exposure paradigm was indeed stressful. Blood samples were collected for plasma corticosterone analysis in male and female mice with or without a history of alcohol self-administration (N= 10–13/group/sex) immediately following the first and fifth stress exposure of the 5-day procedure. Stress significantly elevated plasma corticosterone levels in all groups of mice compared to no-stress controls (Group: [F(1,78)= 187.96, p< 0.001]) and this effect did not habituate over the 5-day stress experience. Overall, naïve mice evidenced higher corticosterone levels than those with a history of alcohol self-administration (History: [F(1,78)= 30.43, p< 0.001]), an effect possibly due to differences in handling. Also, corticosterone levels were higher in females compared to males after stress exposure (Group × History × Sex; [F1,78)= 6.83, p< 0.01]) (Table 1). Separate groups of alcohol-naïve mice sacrificed either 48-hr or 30-days after chronic stress treatment showed elevated plasma corticosterone levels 48-hr after the last stress exposure (Group: [F(1,28)= 29.76, p< 0.0001]) but not at 30-days following the chronic stress regimen (Group: [F(1,28)= 1.20, p= 0.282]) (Table S1).

Another study examined behavioral (emotional) reactivity during each of the five daily consecutive multimodal stress exposure sessions, as measured by defecation. In males (N= 12/group) and females (N= 8–12/group) stress exposure produced overall higher rates of defecation relative to no-stress controls [$F(1,40)= 16.38$, $p< 0.001$]. While the rate of defecation remained elevated across all test days in stress-exposed mice, there was a significant reduction in this stress response over test days in CTL mice (Group × Day interaction: [$F(4,160)= 12.76$, $p< 0.001$]) (Figure 2). This pattern of result was similar for males and females (Group × Day × Sex interaction: [$F4,160)< 1.0$]). In as much as this measure reflects increased emotionality/anxiety (68,69), these data suggest sustained reactivity to the chronic stress procedure over the five days of exposure whereas CTL mice habituated to the novel exposure chamber.

Durability of Sensitized Stress-Induced Alcohol-Seeking Behavior in Mice with a History of Chronic Stress Experience

A separate group of male mice (N= 5/group) was similarly treated and evaluated for stress-induced reinstatement of alcohol-seeking behavior, except testing was conducted at ~30 days and then again at ~60 days following chronic stress (or CTL) exposure (Figure 3A). Baseline responding was similar for the two groups before and after chronic stress (or CTL) exposure as well as following the first reinstatement test session. Replicating our earlier finding, stress (TMT) exposure significantly increased active lever responding (alcohol-seeking) over extinction responding (Test: [$F(1,8)= 46.97$, $p< 0.001$]) and this effect was significantly greater in mice with a history of prior chronic stress exposure (Group × Test interaction: [$F(1,8)= 7.57$, $p= 0.025$]) (Figure 3B). All mice then resumed alcohol self-administration and, after establishing a stable new baseline and a second period of extinction testing, stress (TMT)-induced reinstatement responding was evaluated again. This second reinstatement test session occurred about 60 days following the chronic stress regimen. Acute TMT exposure increased active lever responding above extinction response levels (Test: [$F(1,8)= 53.58$, $p= 0.001$]) and this effect was again significantly greater in mice with a prior history of chronic stress exposure (Group × Test interaction: [$F(1,8)= 9.07$, $p=0.036$]) (Figure 3B and 3C). Thus, in this model chronic multimodal stress exposure enhances the ability of subsequent stress to trigger alcohol relapse-like behavior, and this sensitization is evident for at least 60 days following the chronic stress experience.

Generalizability of Sensitized Stress-Induced Alcohol-Seeking Behavior in Mice with a History of Chronic Stress Experience

To test the generalizability of this sensitized stress-induced alcohol relapse effect, another cohort of male (N= 14–15/group) and female (N= 12–14/group) mice were tested following exposure to environmental cues associated with prior chronic stress (yohimbine+TMT) exposure (Figure 4A). No-stress (CTL) mice were exposed to the unique environmental cues for the first-time during reinstatement testing. Baseline responding before and after chronic Stress/CTL exposure was similar for the groups and both sexes (Group × Sex × Test: [$F(1,51)< 1.0$], although the significant Group × Test interaction [$F(1,51)= 5.58$, $p= 0.022$] indicated an overall lower response rate following the chronic stress treatment (Figures 4B, 4C and Figures S3A, S3B). After extinction responding stabilized, 15-min exposure to the unique context environment increased alcohol-seeking behavior in all mice (Test: [$F(1,51)=$

68.49, p< 0.0001]). However, this effect was significantly greater in male (Figures 4B, 4D) and female (Figures 4C, 4E) mice previously exposed to the multimodal stress experience in that environment compared to the no-stress controls (Group × Test: [F(1,51)= 12.04, p< 0.001]). That is, while increased responding in the CTL group was likely due to the novelty of the environmental cues, exposure to these context cues previously associated with chronic stress exposure resulted in a more robust (sensitized) stress-induced relapse-like response in the Stress group. This effect was similar for males and females as Sex did not significantly interact with either Group or Test variables. These results indicate that exposure to environmental cues previously associated with a chronic stress regimen is sufficient to produce a sensitized stress-induced alcohol relapse-like response.

Effect of Chronic Stress Exposure on Hypothalamic Oxt and Oxtr mRNA Expression.

In alcohol-naïve male and female mice (N= 4–10/group/sex), chronic (5-Day) stress exposure significantly reduced *Oxt* mRNA expression in the hypothalamus 48-hr after the last exposure compared to no-stress controls (Group: [F(1,18)= 8.97, p< 0.01]), and this effect persisted for at least 60 days (Group: [F(1,25)= 8.73,p< 0.01]) (Figure 5A and 5B). The effect was similar in males and females. In contrast, at 48-hr following stress treatment, *Oxtr* mRNA levels in the hypothalamus were significantly elevated relative to controls in males but reduced in females (Figure 5C; Table S2). This was supported by a Group × Sex interaction [F(1,20)= 15.06, p< 0.001], although post-hoc analysis indicated that the reduction in females was not significant. At 60-days post-stress exposure, *Oxtr* mRNA levels were significantly elevated in both males and females (Group: [F(1,25)= 4.69, p< 0.05]). Thus, the chronic multimodal stress procedure in this model produced long-lasting alterations in transcriptional activity of the oxytocin peptide and its receptor in the hypothalamus.

Effect of Oxytocin Treatment on Sensitized Stress-Induced Alcohol-Seeking Behavior in Mice with a History of Chronic Stress Experience

Acute exposure to TMT increased alcohol-seeking in male (N= 12/group) and female (N= 7–12/group) mice, and this effect was significantly enhanced in mice with prior chronic stress experience. This effect in vehicle-treated mice replicates our earlier findings while systemic administration of OT (1 mg/kg) prior to stress exposure completely blocked this TMT-induced alcohol relapse-like response (Figure 6 and Figures S4A and S4B). This was supported by significant main effects of Group (Stress vs. CTL) [F(1,80)= 6.92, p< 0.01], Drug (VEH vs. OT) [F(1,80)= 44.11, p< 0.0001], and Test (Extinction vs. Reinstatement) [F(1,80)= 30.30, p< 0.0001], and the Group × Drug × Test interaction [F(1,80)= 5.90, p< 0.02]. Post-hoc comparisons for vehicle-treated mice indicated that active lever responding was significantly greater during reinstatement testing compared to extinction responding, and this effect was significantly greater in male (Figures 6B and D) and female (Figures 6C and E) mice with a history of chronic stress exposure (ps< 0.05). Treatment with OT prior to reinstatement testing completely blocked stress (TMT)-induced relapse-like responding in both CTL mice and the Stress group (ps< 0.001). ANOVA did not reveal a significant effect of Sex [F(1,80)= 0.73, p> 0.39), and Sex did not interact with any of the other factors. Separate analyses of males and females yielded similar results.

DISCUSSION

Despite the high prevalence and exacerbated problems associated with PTSD and AUD comorbidity, few studies have examined how traumatic stress experience can increase susceptibility to alcohol relapse. The present studies describe a mouse model in which chronic multimodal stress experience enhances the ability of stress to subsequently trigger alcohol relapse-like behavior. This effect was shown to persist for at least 60 days after the chronic stress experience and exposure to only context cues previously associated with the chronic stress experience was sufficient to provoke a sensitized alcohol relapse-like response. As such, this model captures many of the cardinal features of PTSD and links the stress experience with enhanced susceptibility to subsequent stress-provoked alcohol-seeking behavior. Chronic stress exposure also produced long-lasting changes in mRNA levels of oxytocin and its receptor in the hypothalamus. Finally, we demonstrate that systemic administration of OT completely blocked sensitized stress-induced alcohol relapse behavior in mice with a history of chronic stress experience.

Several animal models of PTSD involving exposure to predator odor, including bobcat urine (18,70), soiled cat litter (71), rat bedding (72), and the fox feces extract TMT (20,21) have demonstrated increased alcohol consumption following the stress experience. TMT is known to produce a potent fear/stress response in rodents (73), increasing plasma corticosterone levels in mice (74) and rats (21,75) that is sustained after repeated exposures (20). Here we show that repeated exposure to multimodal stress experience involving combined yohimbine and TMT treatment produced sustained elevated plasma corticosterone levels in mice that persisted for at least 48 hours after the last exposure, and increased defecation that was evident during each of the 5-day exposure sessions. Further, this chronic stress treatment resulted in sensitization to TMT-induced increased alcohol-seeking behavior that was evident at least 60 days later. Predator odor exposure has been shown to increase cue- and stress-induced reinstatement of methamphetamine (76) and cocaine (77) responding. To our knowledge, this is the first evidence demonstrating a PTSD model enhances susceptibility to stress-induced alcohol relapse-like behavior long after the initial stress (‘trauma’) experience.

Context cues associated with predator odor exposure elicit stress-related behavioral responses, and these measures of stress reactivity have been shown to influence subsequent alcohol drinking in rats (18,21,25,70,78). Here we show that exposure to contextual cues previously associated with TMT significantly increased reinstatement of alcohol-seeking responding. Of note, exposure to the stress-related context cues triggered alcohol relapse-like behavior weeks after mice were exposed to TMT in those chambers. These results provide greater face validity for this PTSD model in demonstrating a generalized fear/stress response to environmental cues previously associated with the stressful event, as indicated by the ability of such stress-related cues to enhance alcohol relapse-like behavior.

Oxytocin generally has stress-buffering effects, reducing both physiological and behavioral responses to stress (37). OT is released following stress and signaling at OT receptors in forebrain structures has been shown to modulate stress responses (39,41,42,79–81). Here we show that chronic stress exposure produced a long-lasting reduction in *Oxt* mRNA

that was accompanied by an increase in *Oxtr* mRNA in the hypothalamus. This aligns with reduced hypothalamic *Oxt* mRNA expression reported in male rats following chronic alcohol treatment (54). Stress and alcohol experience have been shown to alter OT and OT receptor transcriptional activity in other brain regions (54,82). It will be interesting to determine in future work whether such changes in projection areas such as the amygdala are revealed in this model, especially since this is a brain site sensitive to neuropeptide modulation of stress-alcohol interactions (83,84). It is also unknown whether a history of alcohol self-administration influences the long-lasting effects of chronic stress on *Oxt* and *Oxtr* mRNA expression in brain.

Systemic administration of OT completely blocked the ability of TMT exposure to trigger alcohol relapse-like behavior. This replicates our earlier findings (58) and extends them to mice demonstrating sensitized stress-induced alcohol-seeking behavior. It is tempting to speculate that OT treatment prior to reinstatement testing mitigates long-term changes in OT activity produced by chronic stress exposure, but this will need to be more directly examined. Also, it will be interesting to determine if OT treatment given more proximal to the chronic stress experience prevents subsequent sensitization to stress in provoking alcohol relapse. While only a single OT dose was evaluated in the present study, we previously showed systemic OT administration to reduce TMT-induced alcohol-seeking behavior in a dose-related manner (58). It is unclear whether higher OT doses may be required to attenuate sensitized stress-induced alcohol relapse resulting from prior chronic stress experience. Nevertheless, these results suggest OT may have therapeutic potential under conditions in which stress triggers alcohol relapse.

Our model of chronic multimodal stress exposure produced similar sensitization to later stress-induced alcohol relapse-like behavior in both males and females. Likewise, at 60 days post-stress exposure, reduced *Oxt* mRNA and elevated *Oxtr* mRNA expression in the hypothalamus was similar in male and female mice. This was somewhat surprising since there is evidence that stress can alter OT expression and activity in a sexually dimorphic manner (85,86). Further, in contrast to an earlier report in male subjects (54), chronic alcohol exposure was not shown to alter *Oxt* mRNA expression in female rat and post-mortem human brain (87). OT expression and signaling in the brain have been shown to be modulated by gonadal hormones (88,89), and future work will be needed to explore potential sex-related differences in the stress-buffering effects of OT, including those relevant to stress-alcohol interactions.

In summary, PTSD and AUD are chronic debilitating disorders, and there is a high prevalence of co-occurrence for these disorders. Unfortunately, few treatments are effective in addressing this significant problem of comorbidity. Further, although stress is known to be a major factor in triggering alcohol relapse, models of PTSD have rarely examined the impact of such stress experiences on later stress-induced alcohol relapse. Here we present a mouse model of PTSD involving chronic multimodal stress experience linked with a well-established model of stress-induced alcohol relapse. Chronic stress exposure sensitized mice to later stress-induced alcohol relapse-like behavior and this effect was evident at least 60 days after the chronic stress experience. Also, the ability of contextual cues previously associated with the chronic stress exposure to trigger relapse was enhanced

long after the chronic stress experience. Thus, this model demonstrates that chronic stress experience increases later vulnerability to alcohol relapse and, in particular, the ability of stress to provoke relapse. Further, chronic stress exposure produced long-lasting alterations in transcriptional activity of OT and OT receptors in the hypothalamus, and systemic administration of OT completely blocked the sensitized stress effect in this model. Together, these data provide support for the potential therapeutic effects of oxytocin in treating stress-related alcohol drinking associated with PTSD-AUD comorbidity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

This work was supported by NIH grants R01 AA026536 (HCB), P50 AA010761 (HCB), U01 AA 014095 (HCB), U24 AA020929 (MFL), F31 AA026483 (CEK) and a grant from the Department of Veterans Affairs (BLRD BX000813) (HCB).

REFERENCES

1. Carlson HN & Weiner JL (2021). The neural, behavioral, and epidemiological underpinnings of comorbid alcohol use disorder and post-traumatic stress disorder. Int Rev Neurobiol 157:69–142. [PubMed: 33648676]
2. Goldstein RB, Smith SM, Chou SP, Saha TD, Jung J, Zhang H, et al. (2016). The epidemiology of DSM-5 posttraumatic stress disorder in the United States: results from the National Epidemiologic Survey on Alcohol and Related Conditions-III. Soc Psychiatry Psychiatr Epidemiol 51(8):1137–1148. [PubMed: 27106853]
3. Grant BF, Goldstein RB, Saha TD, Chou SP, Jung J, Zhang H, et al. (2015). Epidemiology of DSM-5 alcohol use disorder: results From the National Epidemiologic Survey on Alcohol and Related Conditions III. JAMA Psychiatry 72(8):757–766. [PubMed: 26039070]
4. Petrakis IL & Simpson TL (2017). Posttraumatic stress disorder and alcohol use disorder: a critical review of pharmacologic treatments. Alcohol Clin Exp Res 41(2):226–237. [PubMed: 28102573]
5. Smith NDL & Cottler LB (2018). The epidemiology of post-traumatic stress disorder and alcohol use disorder. Alcohol Res 39(2):113–120. [PubMed: 31198651]
6. Dworkin ER, Bergman HE, Walton TO, Walker DD & Kaysen DL (2018). Co-occurring post-traumatic stress disorder and alcohol use disorder in U.S. military and Veteran populations. Alcohol Res 39(2):161–169. [PubMed: 31198655]
7. Seal KH, Cohen G, Waldrop A, Cohen BE, Maguen S & Ren L (2011). Substance use disorders in Iraq and Afghanistan veterans in VA healthcare, 2001–2010: Implications for screening, diagnosis and treatment. Drug Alcohol Depend 116(1–3):93–101. [PubMed: 21277712]
8. Stein MB, Campbell-Sills L, Gelernter J, He F, Heeringa SG, Nock MK, et al. (2017). Alcohol misuse and co-occurring mental disorders among new soldiers in the U.S. army. Alcohol Clin Exp Res 41(1):139–148. [PubMed: 27883222]
9. Dworkin ER, Wanklyn S, Stasiewicz PR & Coffey SF (2018). PTSD symptom presentation among people with alcohol and drug use disorders: Comparisons by substance of abuse. Addict Behav 76:188–194. [PubMed: 28846939]
10. Jacobsen LK, Southwick SM & Kosten TR (2001). Substance use disorders in patients with posttraumatic stress disorder: a review of the literature. Am J Psychiatry 158(8):1184–1190. [PubMed: 11481147]
11. Norman SB, Haller M, Hamblen JL, Southwick SM & Pietrzak RH (2018). The burden of co-occurring alcohol use disorder and PTSD in U.S. military veterans: comorbidities, functioning, and suicidality. Psychol Addict Behav 32(2):224–229. [PubMed: 29553778]

12. Verplaetse TL, McKee SA & Petrakis IL (2018). Pharmacotherapy for co-occurring alcohol use disorder and post-traumatic stress disorder: targeting the opioidergic, noradrenergic, serotonergic, and GABAergic/glutamatergic systems. Alcohol Res 39(2):193–205. [PubMed: 31198658]
13. Deslauriers J, Toth M, Der-Avakian A & Risbrough VB (2018). Current status of animal models of posttraumatic stress disorder: behavioral and biological phenotypes, and future challenges in improving translation. Biol Psychiatry 83(10):895–907. [PubMed: 29338843]
14. Flandreau EI & Toth M (2018). Animal Models of PTSD: A Critical Review. Curr Top Behav Neurosci 38:47–68. [PubMed: 28070873]
15. Gilpin NW & Weiner JL (2017). Neurobiology of comorbid post-traumatic stress disorder and alcohol-use disorder. Genes Brain Behav 16(1):15–43. [PubMed: 27749004]
16. Suh J & Ressler KJ (2018). Common biological mechanisms of alcohol use disorder and post-traumatic stress disorder. Alcohol Res 39(2):131–145. [PubMed: 31198653]
17. Torok B, Sipos E, Pivac N & Zelena D (2019). Modelling posttraumatic stress disorders in animals. Prog Neuropsychopharmacol Biol Psychiatry 90:117–133. [PubMed: 30468906]
18. Edwards S, Baynes BB, Carmichael CY, Zamora-Martinez ER, Barrus M, Koob GF & Gilpin NW (2013). Traumatic stress reactivity promotes excessive alcohol drinking and alters the balance of prefrontal cortex-amygdala activity. Transl Psychiatry 3:e296. doi: 10.1038/tp.2013.70. [PubMed: 23982628]
19. Kirson D, Steinman MQ, Wolfe SA, Spierling Bagsic SR, Bajo M, Sureshchandra S, et al. (2021). Sex and context differences in the effects of trauma on comorbid alcohol use and post-traumatic stress phenotypes in actively drinking rats. J Neurosci Res 99(12):3354–3372. [PubMed: 34687080]
20. Makhijani VH, Franklin JP, Van Voorhies K, Fortino B & Besheer J (2021). The synthetically produced predator odor 2,5-dihydro-2,4,5-trimethylthiazoline increases alcohol self-administration and alters basolateral amygdala response to alcohol in rats. Psychopharmacology (Berl) 238(1):67–82. [PubMed: 32978649]
21. Ornelas LC, Tyler RE, Irukulapati P, Paladugu S & Besheer J (2021). Increased alcohol self-administration following exposure to the predator odor TMT in active coping female rats. Behav Brain Res 402:113068. doi: 10.1016/j.bbr.2020.113068. [PubMed: 33333108]
22. Piggott VM, Lloyd SC, Perrine SA & Conti AC (2020). Chronic intermittent ethanol exposure increases ethanol consumption following traumatic stress exposure in mice. Front Behav Neurosci 14:114. doi: 10.3389/fnbeh.2020.00114. [PubMed: 32694985]
23. Skelly MJ, Chappell AE, Carter E & Weiner JL (2015). Adolescent social isolation increases anxiety-like behavior and ethanol intake and impairs fear extinction in adulthood: Possible role of disrupted noradrenergic signaling. Neuropharmacology 97:149–159. [PubMed: 26044636]
24. Steinman MQ, Kirson D, Wolfe SA, Khom S, D-Ambrosio SR, Spierling Bagsic SR, et al. (2021). Importance of sex and trauma context on circulating cytokines and amygdalar GABAergic signaling in a comorbid model of posttraumatic stress and alcohol use disorders. Mol Psychiatry 26(7):3093–3107. [PubMed: 33087855]
25. Weera MM, Schreiber AL, Avegno EM & Gilpin NW (2020). The role of central amygdala corticotropin-releasing factor in predator odor stress-induced avoidance behavior and escalated alcohol drinking in rats. Neuropharmacology 166:107979. doi: 10.1016/j.neuropharm.2020.107979. [PubMed: 32028150]
26. Koob GF & Schulkin J (2019). Addiction and stress: an allostatic view. Neurosci Biobehav Rev 106:245–262. [PubMed: 30227143]
27. Koob GF & Volkow ND (2016). Neurobiology of addiction: a neurocircuitry analysis. Lancet Psychiatry 3(8):760–773. [PubMed: 27475769]
28. Becker HC (2017). Influence of stress associated with chronic alcohol exposure on drinking. Neuropharmacology 122:115–126. [PubMed: 28431971]
29. Koob GF (2021). Drug addiction: hyperkatifeia/negative reinforcement as a framework for medications development. Pharmacol Rev 73(1):163–201. [PubMed: 33318153]
30. Schank JR, Ryabinin AE, Giardino WJ, Ciccocioppo R & Heilig M (2012). Stress-related neuropeptides and addictive behaviors: beyond the usual suspects. Neuron 76(1):192–208. [PubMed: 23040815]

31. Walker LC (2021). A balancing act: the role of pro- and anti-stress peptides within the central amygdala in anxiety and alcohol use disorders. J Neurochem 157(5):1615–1643. [PubMed: 33450069]
32. Grinevich V, Knobloch-Bollmann HS, Eliava M, Busnelli M & Chini B (2016). Assembling the puzzle: pathways of oxytocin signaling in the brain. Biol Psychiatry 79(3):155–164. [PubMed: 26001309]
33. Grinevich V & Neumann ID (2021). Brain oxytocin: how puzzle stones from animal studies translate into psychiatry. Mol Psychiatry 26(1):265–279. [PubMed: 32514104]
34. Lee MR, Rohn MC, Tanda G & Leggio L (2016). Targeting the oxytocin system to treat addictive disorders: rationale and progress to date. CNS Drugs 30(2):109–123. [PubMed: 26932552]
35. Jurek B & Neumann ID (2018). The oxytocin receptor: from intracellular signaling to behavior. Physiol Rev 98(3):1805–1908. [PubMed: 29897293]
36. Neumann ID & Landgraf R (2012). Balance of brain oxytocin and vasopressin: implications for anxiety, depression, and social behaviors. Trends Neurosci 35(11):649–659. [PubMed: 22974560]
37. Takayanagi Y & Onaka T (2021). Roles of oxytocin in stress responses, allostasis and resilience. Int J Mol Sci 23(1): 150. doi: 10.3390/ijms23010150. [PubMed: 35008574]
38. Giovanna G, Damiani S, Fusar-Poli L, Rocchetti M, Brondino N, de Cagna F, et al. (2020). Intranasal oxytocin as a potential therapeutic strategy in post-traumatic stress disorder: A systematic review. Psychoneuroendocrinology 115:104605. doi: 10.1016/j.psyneuen.2020. [PubMed: 32088633]
39. Knobloch HS, Charlet A, Hoffmann LC, Eliava M, Khrulev S, Cetin AH, et al. (2012). Evoked axonal oxytocin release in the central amygdala attenuates fear response. Neuron 73(3):553–566. [PubMed: 22325206]
40. Le Dorze C, Borreca A, Pignataro A, Ammassari-Teule M & Gisquet-Verrier P (2020). Emotional remodeling with oxytocin durably rescues trauma-induced behavioral and neuro-morphological changes in rats: a promising treatment for PTSD. Transl Psychiatry 10(1):27. [PubMed: 32066681]
41. Martinon D, Lis P, Roman AN, Tornesi P, Applebey SV, Buechner G, Olivera V, et al. (2019). Oxytocin receptors in the dorsolateral bed nucleus of the stria terminalis (BNST) bias fear learning toward temporally predictable cued fear. Transl Psychiatry 9(1):140. doi: 10.1038/s41398-019-0474-x. [PubMed: 31000694]
42. Marvar PJ, Andero R, Hurlemann R, Lago TR, Zelikowsky M & Dabrowska J (2021). Limbic neuropeptidergic modulators of emotion and their therapeutic potential for anxiety and post-traumatic stress disorder. J Neurosci 41(5):901–910. [PubMed: 33472824]
43. Melkonian AJ, Flanagan JC, Calhoun CD, Hogan JN & Back SE (2021). Craving moderates the effects of intranasal oxytocin on anger in response to social stress among Veterans with co-occurring posttraumatic stress disorder and alcohol use disorder. J Clin Psychopharmacol 41(4):465–469. [PubMed: 34121063]
44. Sippel LM, King CE, Wahlquist AE & Flanagan JC (2020). A preliminary examination of endogenous peripheral oxytocin in a pilot randomized clinical trial of oxytocin-enhanced psychotherapy for posttraumatic stress disorder. J Clin Psychopharmacol 40(4):401–404. [PubMed: 32639293]
45. van Zuiden M, Frijling JL, Nawijn L, Koch SB, Goslings JC, Luitse JS, et al. (2016). Intranasal oxytocin to prevent posttraumatic stress disorder symptoms: a randomized controlled trial in emergency department patients. Biol Psychiatry 81(12):1030–1040. [PubMed: 28087128]
46. Bowen MT & Neumann ID (2018). The multidimensional therapeutic potential of targeting the brain oxytocin system for the treatment of substance use disorders. Curr Top Behav Neurosci 35:269–287. [PubMed: 28942596]
47. King CE, Gano A & Becker HC (2020). The role of oxytocin in alcohol and drug abuse. Brain Res 1736:146761. doi: 10.1016/j.brainres.2020. [PubMed: 32142721]
48. Lee MR & Weerts EM (2016). Oxytocin for the treatment of drug and alcohol use disorders. Behav Pharmacol 27(8):640–648. [PubMed: 27603752]
49. Ryabinin AE & Fulenwider HD (2021). Alcohol and oxytocin: scrutinizing the relationship. Neurosci Biobehav Rev 127:852–864. [PubMed: 34102150]

50. Ryabinin AE & Zhang Y (2022). Barriers and breakthroughs in targeting the oxytocin system to treat alcohol use disorder. Front Psychiatry 13:842609. doi: 10.3389/fpsyt.2022.842609. [PubMed: 35295777]
51. Pedersen CA, Smedley KL, Leserman J, Jarskog LF, Rau SW, Kampov-Polevoi A, et al. (2013). Intranasal oxytocin blocks alcohol withdrawal in human subjects. Alcohol Clin Exp Res 37(3):484–489. [PubMed: 23025690]
52. Mitchell JM, Arcuni PA, Weinstein D & Woolley JD (2016). Intranasal oxytocin selectively modulates social perception, craving, and approach behavior in subjects with alcohol use disorder. J Addict Med 10(3):182–189. [PubMed: 27159342]
53. Bach P, Reinhard I, Buhler S, Vollstadt-Klein S, Kiefer F & Koopmann A (2019). Oxytocin modulates alcohol-cue induced functional connectivity in the nucleus accumbens of social drinkers. Psychoneuroendocrinology 109:104385. doi: 10.1016/j.psyneuen.2019. [PubMed: 31362183]
54. Hansson AC, Koopmann A, Uhrig S, Buhler S, Domi E, Kiessling E, et al. (2018). Oxytocin reduces alcohol cue-reactivity in alcohol-dependent rats and humans. Neuropsychopharmacology 43(6):1235–1246. [PubMed: 29090683]
55. Melby K, Grawe RW, Aamo TO, Skovlund E & Spigset O (2021). Efficacy of self-administered intranasal oxytocin on alcohol use and craving after detoxification in patients with alcohol dependence. a double-blind placebo-controlled trial. Alcohol Alcohol 56(5):565–572. [PubMed: 33352584]
56. Vena A, King A, Lee R & de Wit H (2018). Intranasal oxytocin does not modulate responses to alcohol in social drinkers. Alcohol Clin Exp Res 42(9):1725–1734. [PubMed: 29917245]
57. Caruso MA, Robins MT, Fulenwider HD & Ryabinin AE (2021). Temporal analysis of individual ethanol consumption in socially housed mice and the effects of oxytocin. Psychopharmacology (Berl) 238(3):899–911. [PubMed: 33404737]
58. King CE & Becker HC (2019). Oxytocin attenuates stress-induced reinstatement of alcohol seeking behavior in male and female mice. Psychopharmacology (Berl) 236(9):2613–2622. [PubMed: 30923836]
59. King CE, Griffin WC, Lopez MF & Becker HC (2021). Activation of hypothalamic oxytocin neurons reduces binge-like alcohol drinking through signaling at central oxytocin receptors. Neuropsychopharmacology 46(11):1950–1957. [PubMed: 34127796]
60. King CE, Griffin WC, Luderman LN, Kates MM, McGinty JF & Becker HC (2017). Oxytocin reduces ethanol self-administration in mice. Alcohol Clin Exp Res 41(5):955–964. [PubMed: 28212464]
61. MacFadyen K, Loveless R, DeLucca B, Wardley K, Deogan S, Thomas G & Peris J (2016). Peripheral oxytocin administration reduces ethanol consumption in rats. Pharmacol Biochem Behav 140:27–32. [PubMed: 26519603]
62. Peters ST, Bowen MT, Bohrer K, McGregor IS & Neumann ID (2017). Oxytocin inhibits ethanol consumption and ethanol-induced dopamine release in the nucleus accumbens. Addict Biol 22(3):702–711. [PubMed: 26810371]
63. Stevenson JR, Wenner SM, Freestone DM, Romaine CC, Parian MC, Christian SM, et al. (2017). Oxytocin reduces alcohol consumption in prairie voles. Physiol Behav 179:411–421. [PubMed: 28716609]
64. Tunstall BJ, Kirson D, Zallar LJ, McConnell SA, Vendruscolo JCM, Ho CP, et al. (2019). Oxytocin blocks enhanced motivation for alcohol in alcohol dependence and blocks alcohol effects on GABAergic transmission in the central amygdala. PLoS Biol 17(4):e2006421. doi: 10.1371/journal.pbio.2006421. [PubMed: 30990816]
65. Walcott AT & Ryabinin AE (2021). Assessing effects of oxytocin on alcohol consumption in socially housed prairie voles using radio frequency tracking. Addict Biol 26(2):e12893. doi: 10.1111/adb.12893. [PubMed: 32160654]
66. Haun HL, Lebonville CL, Solomon MG, Griffin WC, Lopez MF & Becker HC (2022). Dynorphin/Kappa opioid receptor activity within the extended amygdala contributes to stress-enhanced alcohol drinking in mice. Biol Psychiatry 91(12):1019–1028. [PubMed: 35190188]

67. Solomon MG, Griffin WC, Lopez MF & Becker HC (2019). Brain regional and temporal changes in BDNF mRNA and microRNA-206 expression in mice exposed to repeated cycles of chronic intermittent ethanol and forced swim stress. Neuroscience 406:617–625. [PubMed: 30790666]
68. Ayash S, Schmitt U, Lyons DM & Muller MB (2020). Stress inoculation in mice induces global resilience. Transl Psychiatry 10(1):200. doi: 10.1038/s41398-020-00889-0. [PubMed: 32561821]
69. Julio-Pieper M, O'Mahony CM, Clarke G, Bravo JA, Dinan TG & Cryan JF (2012). Chronic stress-induced alterations in mouse colonic 5-HT and defecation responses are strain dependent. Stress 15(2):218–226. [PubMed: 21875301]
70. Albrechet-Souza L & Gilpin NW (2019). The predator odor avoidance model of post-traumatic stress disorder in rats. Behav Pharmacol 30(2 and 3-Spec Issue):105–114. [PubMed: 30640179]
71. Manjoch H, Vainer E, Matar M, Ifergane G, Zohar J, Kaplan Z & Cohen H (2016). Predator-scent stress, ethanol consumption and the opioid system in an animal model of PTSD. Behav Brain Res 306:91–105. [PubMed: 26965572]
72. Finn DA, Helms ML, Nipper MA, Cohen A, Jensen JP & Devaud LL (2018). Sex differences in the synergistic effect of prior binge drinking and traumatic stress on subsequent ethanol intake and neurochemical responses in adult C57BL/6J mice. Alcohol 71:33–45. [PubMed: 29966824]
73. Rosen JB, Asok A & Chakraborty T (2015). The smell of fear: innate threat of 2,5-dihydro-2,4,5-trimethylthiazoline, a single molecule component of a predator odor. Front Neurosci 9:292. doi: 10.3389/fnins.2015.00292. [PubMed: 26379483]
74. Chan CE, Lee YU & Swoap SJ (2021). Physiological response to the odorant TMT in fully fed and calorically restricted laboratory mice. J Therm Biol 95:102819. doi: 10.1016/j.jtherbio.2020.102819. [PubMed: 33454047]
75. Endres T & Fendt M (2009). Aversion- vs fear-inducing properties of 2,4,5-trimethyl-3-thiazoline, a component of fox odor, in comparison with those of butyric acid. J Exp Biol 212(Pt 15):2324–2327. [PubMed: 19617424]
76. Ferland CL, Reichel CM & McGinty JF (2016). Effects of oxytocin on methamphetamine-seeking exacerbated by predator odor pre-exposure in rats. Psychopharmacology (Berl) 233(6):1015–1024. [PubMed: 26700240]
77. Schwendt M, Shallcross J, Hadad NA, Namba MD, Hiller H, Wu L, et al. (2018). A novel rat model of comorbid PTSD and addiction reveals intersections between stress susceptibility and enhanced cocaine seeking with a role for mGlu5 receptors. Transl Psychiatry 8(1):209. doi: 10.1038/s41398-018-0265-9. [PubMed: 30291225]
78. Ornelas LC, Van Voorhies K & Besheer J (2021). The role of the nucleus reuniens in regulating contextual conditioning with the predator odor TMT in female rats. Psychopharmacology (Berl) 238(12):3411–3421. [PubMed: 34390359]
79. Ebner K, Bosch OJ, Kromer SA, Singewald N & Neumann ID (2005). Release of oxytocin in the rat central amygdala modulates stress-coping behavior and the release of excitatory amino acids. Neuropsychopharmacology 30(2):223–230. [PubMed: 15536493]
80. Hasan MT, Althammer F, Silva da Gouveia M, Goyon S, Eliava M, Lefevre A, et al. (2019). A fear memory engram and its plasticity in the hypothalamic oxytocin system. Neuron 103(1):133–146.e8. [PubMed: 31104950]
81. Viviani D, Charlet A, van den Burg E, Robinet C, Hurni N, Abatis M, et al. (2011). Oxytocin selectively gates fear responses through distinct outputs from the central amygdala. Science 333(6038):104–107. [PubMed: 21719680]
82. Barchiesi R, Chanthongdee K, Domi E, Gobbo F, Coppola A, Asratian A, et al. (2021). Stress-induced escalation of alcohol self-administration, anxiety-like behavior, and elevated amygdala Avp expression in a susceptible subpopulation of rats. Addict Biol 26(5):e13009. doi: 10.1111/adb.13009. [PubMed: 33565224]
83. Ballas HS, Wilfur SM, Freker NA & Leong KC (2021). Oxytocin attenuates the stress-induced reinstatement of alcohol-seeking in male rats: role of the central amygdala. Biomedicines 9(12):1919. doi: 10.3390/biomedicines9121919. [PubMed: 34944734]
84. Haun HL, Lebonville CL, Solomon MG, Griffin WC, Lopez MF & Becker HC (2022). Dynorphin/Kappa opioid receptor activity within the extended amygdala contributes to stress-enhanced alcohol drinking in mice. Biol Psychiatry 91(12):1019–1028. [PubMed: 35190188]

85. Bisagno V & Cadet JL (2014). Stress, sex, and addiction: potential roles of corticotropin-releasing factor, oxytocin, and arginine-vasopressin. Behav Pharmacol 25(5–6):445–457. [PubMed: 24949572]

86. Steinman MQ, Duque-Wilckens N, Greenberg GD, Hao R, Campi KL, Laredo SA, et al. (2016). Sex-specific effects of stress on oxytocin neurons correspond with responses to intranasal oxytocin. Biol Psychiatry 80(5):406–414. [PubMed: 26620251]

87. Hansson AC & Spanagel R (2021). No changes in the oxytocin system in alcohol-dependent female rodents and humans: towards a sex-specific psychopharmacology in alcoholism. Addict Biol 26(2):e12945. doi: 10.1111/adb.12945. [PubMed: 32761675]

88. Dumais KM, Bredewold R, Mayer TE & Veenema AH (2013). Sex differences in oxytocin receptor binding in forebrain regions: correlations with social interest in brain region- and sex- specific ways. Horm Behav 64(4):693–701. [PubMed: 24055336]

89. Dumais KM & Veenema AH (2016). Vasopressin and oxytocin receptor systems in the brain: sex differences and sex-specific regulation of social behavior. Front Neuroendocrinol 40:1–23. doi: 10.1016/j.yfrne.2015.04.003. [PubMed: 25951955]

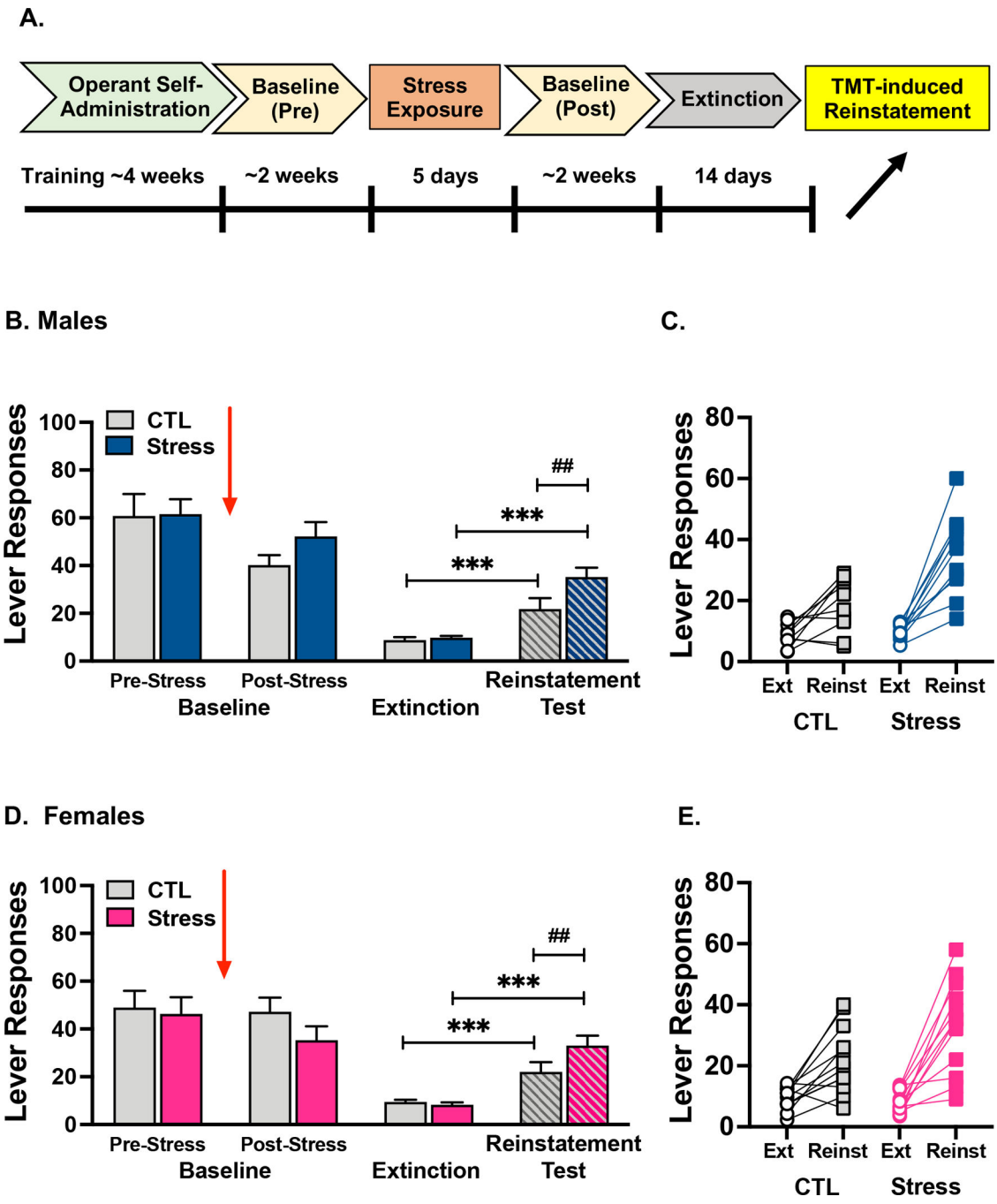

Figure 1: Sensitized Stress-Induced Alcohol Relapse-Like Behavior in Mice with a History of Chronic Stress Exposure.

(A) Experimental procedures and timeline for alcohol self-administration, chronic stress exposure, extinction testing, and reinstatement testing phases of the study. Lever responses for **(B, C)** males and **(D, E)** females during each phase of the study for mice in the chronic stress group or no-stress control (CTL) condition. Red arrow denotes 5-day stress exposure period. Values are mean ± s.e.m. active lever responses during baseline alcohol self-administration (last 5 days prior to chronic stress exposure and last 5-days prior to

extinction testing), the last 5 days of extinction responding, and responses during the reinstatement test. Acute predator odor (TMT) exposure significantly increased alcohol-seeking behavior in CTL male and female mice above their level of responding during extinction, and this effect was significantly greater in mice with a history of chronic stress exposure. *** significantly differs from respective extinction responding (ps< 0.001); ## significantly differs from TMT-induced reinstatement responding in CTL group (p< 0.01).

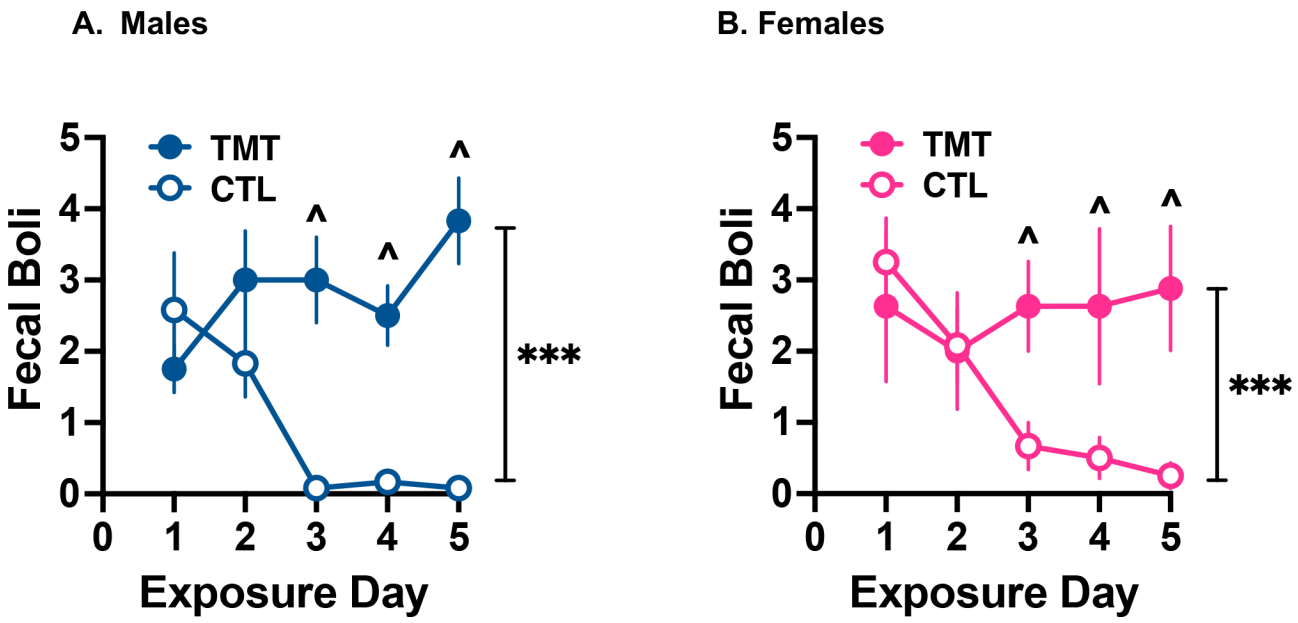

Figure 2: Sustained Stress/Emotional Reactivity During Repeated Stress Exposure Sessions.

Mean ± s.e.m. defecation droppings for **(A)** male and **(B)** female mice during each of five daily consecutive stress exposure sessions. Stress-exposed mice received yohimbine (2 mg/kg) 15-min being placed in a chamber for 15-min exposure to TMT. CTL mice were injected with saline and then placed in a control chamber.. *** significantly differs from control group (main effect of TMT exposure; ps< 0.001); ^ significantly differs from control group at each respective exposure session (ps< 0.01).

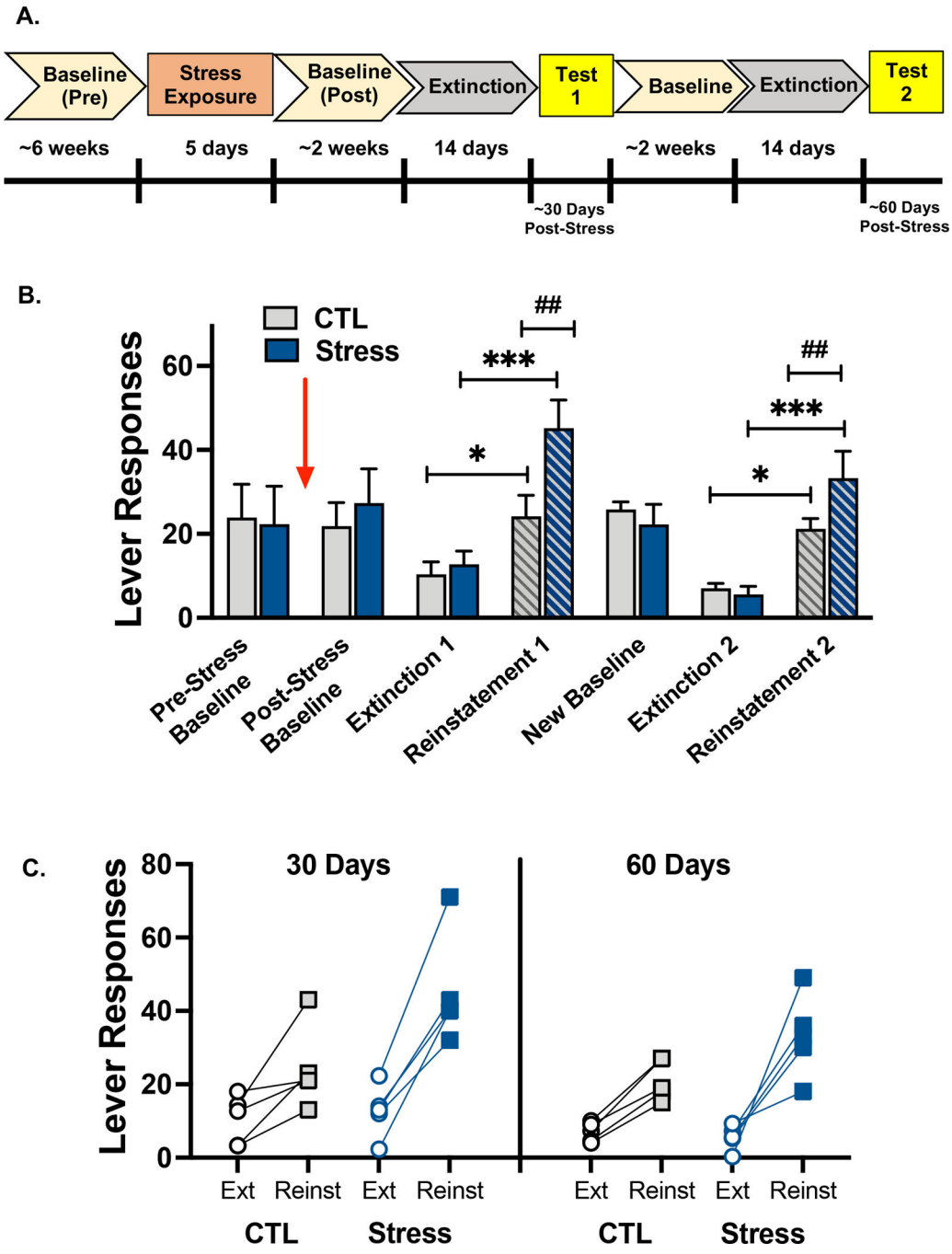

Figure 3: Long-Lasting Sensitization of Stress-Induced Alcohol Relapse-Like Behavior in Mice with a History of Chronic Stress Exposure.

(A) Experimental procedures and timeline for alcohol self-administration, chronic stress exposure, extinction testing, and reinstatement testing phases of the study. **(B)** Mean ± s.e.m. active lever responses during each phase of the study for male mice in the chronic stress group or no-stress control (CTL) condition. Red arrow denotes 5-day multimodal stress exposure period. During the first reinstatement test, acute TMT exposure significantly increased alcohol relapse-like responding in both CTL and Stress mice above

their respective levels of extinction responding, and this effect was significantly greater in mice with a history of chronic stress exposure. After re-establishing stable baseline alcohol self-administration followed by extinction responding, the same profile of results was observed during a second reinstatement test, which occurred about 60 days following chronic stress exposure. * significantly differs from CTL group extinction responding (ps< 0.05); ***significantly differs from Stress group extinction responding (ps< 0.001); ## significantly differs from TMT-induced reinstatement responding in CTL group (ps< 0.01). **(C)** Lever responding for individual male mice in Stress and CTL groups during the final extinction session preceding stress (TMT)-induced reinstatement testing at ~30 days and ~60 days following the 5-day chronic stress treatment.

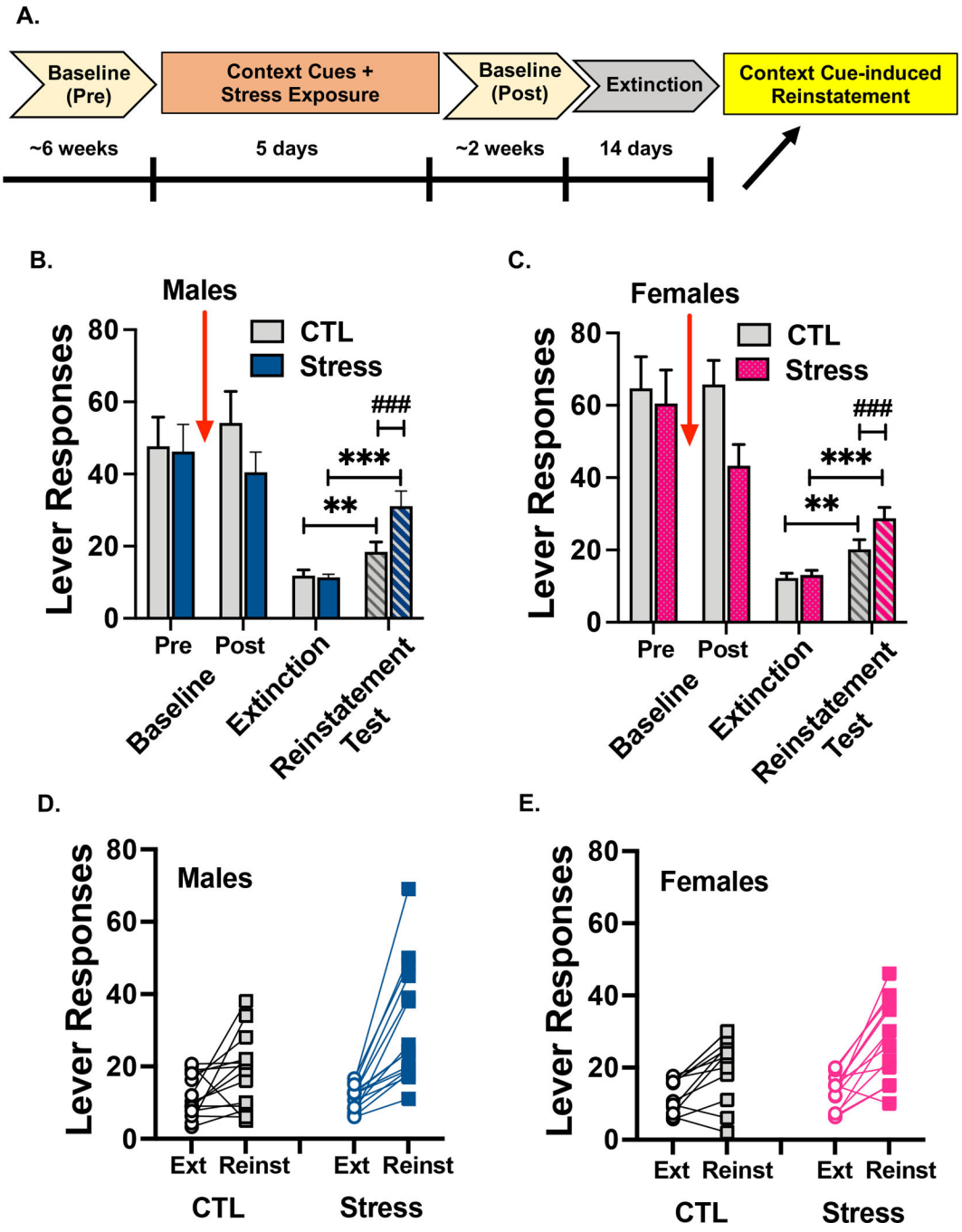

Figure 4: Exposure to Context Cues Previously Associated with Chronic Stress Enhances Alcohol Relapse-Like Behavior in Male and Female Mice.

(A) Experimental procedures and timeline for alcohol self-administration, chronic stress exposure, extinction testing, and reinstatement testing phases of the study. Note: during reinstatement testing mice were exposed to environmental (context) cues in the absence of TMT. Mean ± s.e.m. lever responses for **(B)** male and **(C)** female mice during each phase of the study for Stress and CTL groups. Red arrow denotes 5-day yohimbine+TMT exposure in unique environmental context. Male and female CTL mice exposed to the environmental

cues for the first time prior to the reinstatement test session showed significantly increased alcohol-seeking behavior while male and female mice exposed to the context cues previously associated with chronic stress exposure (Stress group) evidenced a significantly greater alcohol relapse-like response. ** significantly differs from CTL group extinction responding (ps< 0.01); *** significantly differs from Stress group extinction responding (ps< 0.001); ### significantly differs from stress-induced reinstatement responding in CTL group (ps< 0.001). Lever responding for individual **(D)** male and **(E)** female mice in Stress and CTL groups during the final extinction session preceding context cue-induced reinstatement testing.

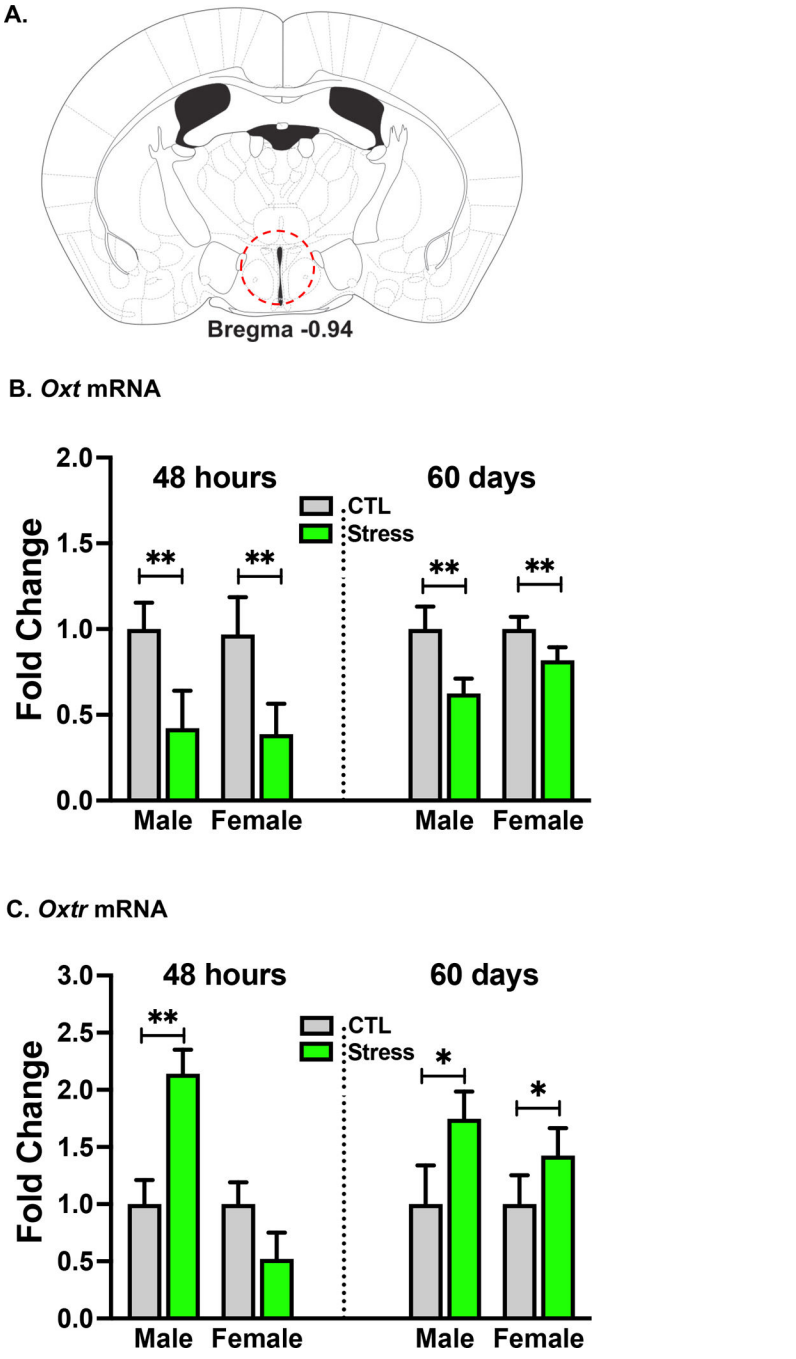

Figure 5: Chronic Stress Exposure Produces Long-Lasting Changes Hypothalamic Oxt and Oxtr mRNA Expression.

(A) Schematic representation of tissue punch used for determination of transcriptional changes in oxytocin and its receptor in the hypothalamus. Mice were sacrificed and brain tissue was collected at 48-hr or 60-days following chronic (5-day) stress exposure. **(B)** *Oxt* mRNA expression in the hypothalamus of alcohol-naïve male and female mice was significantly reduced 48-hr following the final stress exposure relative to no-stress controls, and this reduction persisted for at least 60-days following the chronic stress treatment.

** significantly differs from CTL group (ps< 0.01). **(C)** *Oxtr* mRNA expression in the hypothalamus was significantly elevated in male mice but reduced (trend) in females relative to the controls at 48-hr following the chronic stress procedure. ** significantly differs from respective CTL group (p< 0.01). *Oxtr* mRNA levels were significantly elevated in both male and female mice relative to CTL levels at 60-days post-stress treatment. * significantly differs from respective CTL group (ps< 0.05).

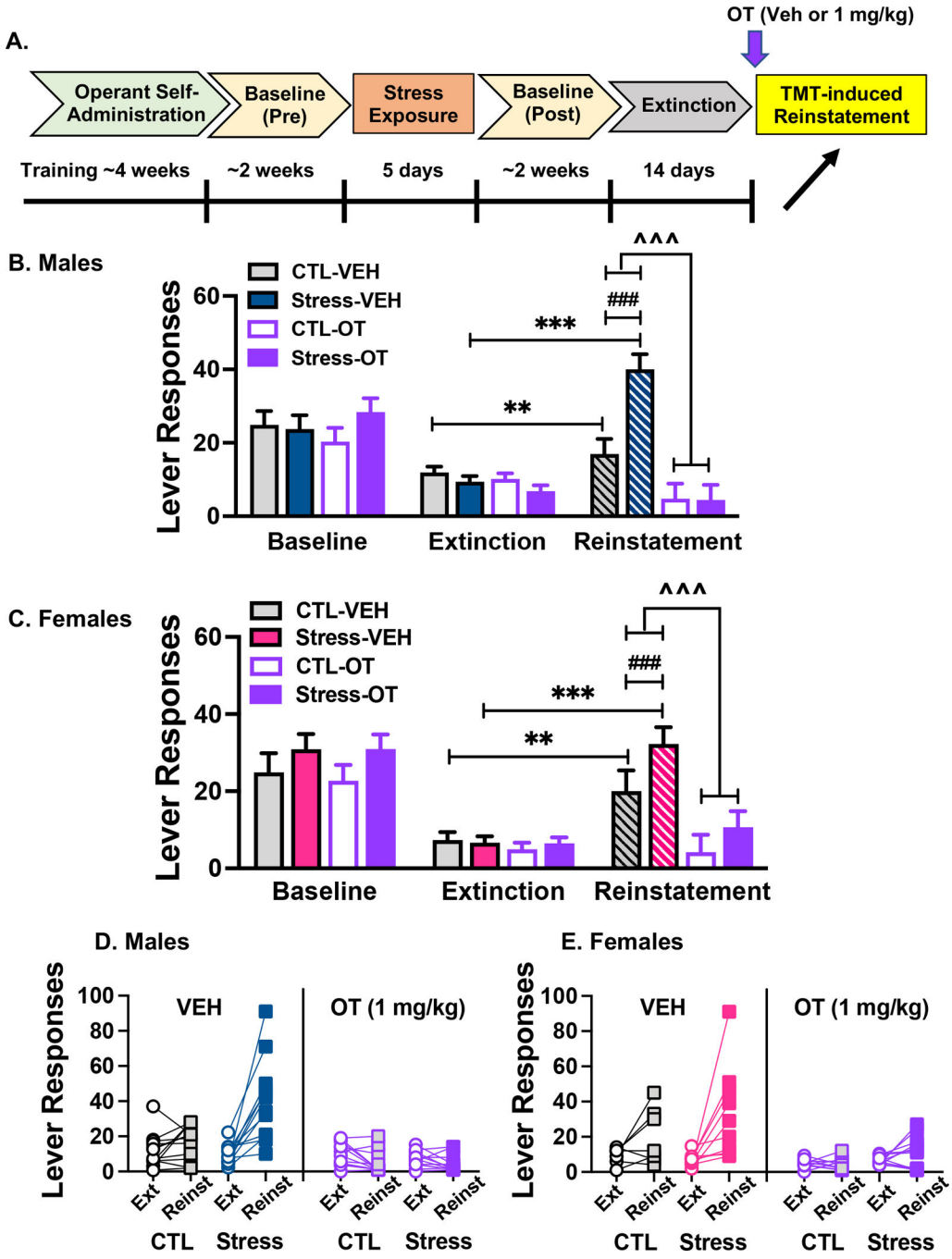

Figure 6: Oxytocin Blocks Sensitized Stress-Induced Alcohol Relapse-Like Behavior in Mice with a History of Chronic Stress Exposure.

(A) Experimental procedures and timeline for alcohol self-administration, chronic stress exposure, extinction testing, and reinstatement testing phases of the study. Mean ± s.e.m. lever responses for **(B)** male and **(C)** female mice during each phase of the study for Stress and CTL groups treated with OT (1 mg/kg) or Vehicle prior to reinstatement testing. In Vehicle-treated CTL male and female mice, acute TMT exposure significantly increased alcohol-seeking behavior above their level of responding during extinction, and this effect

was significant greater in mice with a history of chronic stress exposure. ** significantly differs from extinction responding in CTL-Veh group ($p < 0.01$); *** significantly differs from extinction responding in Stress-Veh group ($p < 0.001$); ### significantly differs from TMT-induced reinstatement responding in CTL group ($p < 0.001$). OT blocked TMT-induced alcohol-seeking behavior in CTL and Stress male and female groups. ^^^ significantly differs from Vehicle-treated CTL and Stress groups ($p < 0.001$). Lever responding for individual **(D)** male and **(E)** female mice in Stress and CTL groups during the final extinction session prior to reinstatement testing when mice were pretreated with Vehicle or OT (1 mg/kg).

Table 1:

Plasma Corticosterone Levels (ug/dL) Immediately Following the First and Fifth Day of Stress Exposure in Male and Female Mice With or Without a History of Alcohol Self-Administration.

		Males		Females	
		Day-1	**Day-5**	**Day-1**	**Day-5**
Naive	**CTL**	21.74 ± 1.03	23.88 ± 1.91	27.51 ± 0.67	24.90 ± 1.37
	Stress	30.28 ± 1.62 *	34.27 ± 1.56 *	35.50 ± 1.62 *	36.21 ± 1.59 *
Alcohol SA	**CTL**	16.64 ± 1.05	16.34 ± 0.76	13.61 ± 1.42	21.56 ± 1.85
	Stress	25.75 ± 1.41 *	27.24 ± 1.68 *	33.14 ± 1.42 *	40.94 ± 1.41 *

Values are mean ± s.e.m. (N= 10–13/group/sex).

* differs from corresponding CTL value ($p < 0.01$).

KEY RESOURCES TABLE

Resource Type	Specific Reagent or Resource	Source or Reference	Identifiers	Additional Information
Add additional rows as needed for each resource type	Include species and sex when applicable.	Include name of manufacturer, company, repository, individual, or research lab. Include PMID or DOI for references; use “this paper” if new.	Include catalog numbers, stock numbers, database IDs or accession numbers, and/or RRIDs. RRIDs are highly encouraged; search for RRIDs at https://scicrunch.org/resources.	Include any additional information or notes if necessary.
Antibody	N/A			
Bacterial or Viral Strain	N/A			
Biological Sample	Mouse brain	This study		
Cell Line	N/A			
Chemical Compound or Drug	2,3,5-Trimethyl-3-thiazoline (TMT)	BioSRQ		
Chemical Compound or Drug	yohombine	Tocris Bioscience	Catalog No. 1127	
Chemical Compound or Drug	oxytocin	Cell sciences	Catalog No. CRO300A	
Commercial Assay Or Kit	corticosterone ELISA	Arbor Assays	Catalog No. K014-H5	
Commercial Assay Or Kit	RNA Extraction Kit	Promega	Catalog No. Z6212	
Commercial Assay Or Kit	QuantiTect Reverse Transcription Kit	Qiagen	Catalog No. 205314	
Deposited Data; Public Database	N/A			
Genetic Reagent	N/A			
Organism/Strain	Mouse, C57BL/6J, males and females	Jackson Laoratories	RRID:IMSR_JAX:000664	
Peptide, Recombinant Protein	N/A			
Recombinant DNA	N/A			
Sequence-Based Reagent	TaqMan qRT-PCR primer (Oxt)	ThermoFisher Scientific	Mm01329577_g1	
Sequence-Based Reagent	TaqMan qRT-PCR primer (Oxtr)	ThermoFisher Scientific	Mm07308231_u1	
Sequence-Based Reagent	TaqMan qRT-PCR primer (Ppia)	ThermoFisher Scientific	Mm02342430_gl	
Software; Algorithm	N/A			
Transfected Construct	N/A			
Other				