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Oxytocin Attenuates Stress-Induced Reinstatement of Alcohol Seeking Behavior in Male and Female Mice

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Abstract

Rationale: The neuropeptide oxytocin (OXT) has emerged as a potential therapeutic intervention in the treatment of both alcohol use disorder (AUD) and stress-related psychiatric illnesses.

Objectives: The present study evaluates the effects of systemically administered (intraperitoneal, i.p.) OXT treatment on alcohol relapse-like behavior in male and female mice.

Methods: Adult male and female C57BL/6J mice were trained to lever respond in operant conditioning chambers for alcohol in daily self-administration sessions. Once lever responding and alcohol intake stabilized mice were tested under extinction conditions for 14 days before reinstatement testing. All mice underwent stress-induced reinstatement testing using either predator odor (2,3,5-Trimethyl-3-thiazoline; TMT) or the α−2 adrenergic receptor agonist yohimbine. In Study 1, mice were exposed to TMT for 15 min and then immediately placed into operant conditioning chambers to examine alcohol-seeking behavior under extinction conditions. At 30 min prior to test session, separate groups of mice were injected with vehicle or OXT (0.1, 0.5, 1 mg/kg). In Study 2, mice were injected with yohimbine (0.3, 0.625 mg/kg) 1 hr prior to reinstatement testing. At 30 min post-yohimbine injection, mice are injected (ip.) with vehicle or OXT (1 mg/kg).

Results: OXT attenuated alcohol-seeking behavior in a dose-related manner in male and female mice in response to acute challenge with a predator odor. Additionally, OXT administration produced a similar decrease in alcohol relapse-like behavior triggered by the pharmacological stressor yohimbine in both sexes.

Conclusions: Systemic oxytocin administration attenuates stress-induced reinstatement of alcohol seeking in male and female mice.

Keywords

Oxytocin; Alcohol; Stress; Relapse; Self-Administration; Mouse

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INTRODUCTION

The compulsive nature of alcohol seeking and use, along with high rates of relapse present a major challenge in the treatment of alcohol addiction. Stress is a common trigger for relapse, yet mechanisms by which stress provokes relapse and promotes excessive levels of alcohol consumption are not fully understood. Thus, understanding brain mechanisms underlying relapse and the influence of stress on drinking has emerged as a central issue in addiction research.

Oxytocin (OXT) is an endogenous neuropeptide that has been implicated in various processes associated with addiction, including reward, tolerance, memory and stress responses (Lee et al. 2016). OXT is synthesized in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus and released by the posterior pituitary into peripheral circulation. In addition, OXT is released by neurons in the PVN that project to numerous extrahypothalamic regions in the brain (e.g., cortical, limbic, basal ganglia structures) where it mediates an array of behavioral effects via interaction with G(q)-coupled OXT receptors (Lee et al. 2016). Aside from its known hormonal role in parturition and maternal behaviors, OXT also regulates a number of behaviors that involve social interaction (e.g., pair-bonding, social reward processing, aggression) and nonsocial behaviors, including anxiety and response to stress (Baskerville and Douglas 2010; Burkett et al. 2016; Neumann and Landgraf 2012). Through interactions with brain reward and stress systems, OXT is known to play a role in several neuropsychiatric disorders that involve social deficits, including drug and alcohol addiction (Baskerville and Douglas 2010; Lee et al. 2016).

Understandably, the ability of this nonapeptide to modulate both stress and motivational processes has generated growing interest in its potential as a treatment for substance use disorders. For example, in a recent clinical study, intranasal OXT was shown to alleviate alcohol withdrawal symptoms in treatment-seeking subjects (Pedersen et al. 2013). Additional support comes from a growing number of studies showing that systemic administration of OXT reduces alcohol consumption in rodents (Bowen et al. 2011; MacFadyen et al. 2016; McGregor and Bowen 2012). Further, direct brain (intracerebroventricular) infusion of OXT, or manipulation of OXT receptor expression in brain reduced alcohol reward and consumption (Bahi 2015; Bahi et al. 2016; Peters et al. 2017). Recent work in our laboratory demonstrated that systemic administration of OXT reduced binge-like alcohol drinking and oral self-administration of alcohol using operant conditioning procedures in male C57BL/6J mice in a dose-related manner (King et al. 2017). Importantly, OXT reduced alcohol consumption at doses that did not significantly alter sucrose intake. Taken together, accumulating evidence suggests that the OXT system may represent a promising target for the treatment of alcohol use disorders.

While evidence supports a role for OXT in regulating alcohol consumption, few studies have examined the effects of OXT on alcohol relapse-like behavior. The present study aimed to examine the ability of systemic oxytocin to attenuate stress-induced reinstatment of alcohol-seeking behavior in male and female mice. Two different stress procedures were employed: brief exposure to a predator odor and acute challenge with the pharmacological stressor yohimbine. Given that OXT is know to function as an anti-stress neuropeptide (Landgraf and

Neumann 2004; Windle et al. 2004), it was predicted that systemic OXT administration would attenuate stress-induced reinstatement of alcohol-seeking behavior in a dose-related manner in male and female mice. Further, since there is some evidence to suggest that gonadal hormones can influence the functional activity of OXT (Ivell and Walther 1999), it was predicted that females would exhibit a greater response to OXT treatment compared to males.

MATERIALS AND METHODS

Subjects

Adult male and female C57BL/6J mice (25 to 30 g) were obtained from Jackson Laboratories (Bar Harbor, ME) at 9 to 10 weeks of age and were acclimated to the experimental housing rooms for a minimum of 1 week prior to the start of experiments. Male and female mice were group housed (4 per cage) in the same colony room, but with males and females on separate racks. The animals were maintained under a 12-hour reversed light/dark cycle in an AAALAC-accredited facility. All testing was conducted during the dark phase of the circadian cycle. Mice were provided free access to food and water, except at the start of operant oral self-administration training. All experimental protocols were approved by the Medical University of South Carolina Institutional Animal Care and Use Committee and consistent with guidelines of the NIH Guide for the Care and Use of Laboratory Animals.

Operant Conditioning Chambers

Mice were tested in standard operant conditioning chambers configured with 2 retractable levers, a centrally located well for liquid reinforcers, house light, a tone generator, and a stimulus light above the well. The chambers were situated in sound-attenuating cubicles with a ventilation fan (Med Associates; Fairfax, VT). One lever was assigned as the “active” lever, with scheduled responses activating an infusion pump that delivered alcohol (12% v/v ethanol) reinforcement (20 μl) into the well along with activating a light and a tone stimulus (80 dB). Responses on the “inactive lever” were recorded, but did not result in any stimulus consequences or reinforcement delivery. The position of the “active” lever (left vs. right of well) was counterbalanced across subjects. Stimulus events and responses were controlled and monitored using Med PC, Version IV software (Med Associates). At the end of each session, any residual fluid left in the well was collected with a pipette, measured, and subtracted from the total volume of alcohol delivered. The corrected volume was used to calculate g/kg alcohol intake during the 20-min daily sessions. Male and female mice were tested in separate chambers throughout the experiments (8 chambers/sex).

Operant Alcohol Self-Administration

Mice were trained to self-administer alcohol using standard operant conditioning procedures, as previously described (King et al. 2017). Briefly, mice were first trained to respond for alcohol reinforcement under a fixed ratio-1 (FR1) schedule. Response requirements were gradually increased until mice achieved stable lever responding under a FR4 schedule of reinforcement (<15% variability across 3 consecutive daily sessions). Once stable baseline alcohol responding/intake was established, mice entered into the extinction

phase of the study. During daily extinction sessions, responding on the “active” lever no longer had stimulus consequences and alcohol reinforcement was no longer delivered. After 14 days of extinction (when responding has reached <15% of baseline responding), all mice were tested for stress-induced reinstatement of alcohol responding.

Study 1: Predator Odor-Induced Reinstatement Testing

Mice were transferred to a chamber and exposed to a predator odor (2,3,5-Trimethyl-3-thiazoline; TMT) for 15 min. This stressor, a synthetically derived component of fox feces, was selected because of its ethological relevance and validity (Janitzky et al. 2015), as well as the fact that we have successfully used TMT to produce robust relapse-like behavior in our preliminary studies. TMT (0.03 mL; 1% v/v) was placed on a gauze pad in a weigh boat in the chamber such that mice could not manipulate it. After the 15-min exposure period, mice were immediately placed into self-administration chambers to examine alcohol-seeking behavior under extinction conditions. At 30 min prior to the reinstatement test session (15 min prior to TMT exposure), separate groups of mice were injected (ip.) with vehicle (saline) or OXT (0.1, 0.5, 1 mg/kg) (N= 8–10/group).

Study 2: Yohimbine-Induced Reinstatement Testing

Mice were injected with yohimbine (0.3, 0.625 mg/kg; ip.) 60 min prior to the reinstatement test session. At 30 min post-yohimbine injection, mice are injected (ip.) with vehicle (saline) or OXT (1 mg/kg). Reinstatement testing was conducted under extinction conditions 30 min after OXT injection. Results from a series of pilot studies indicated that the 60 min pretreatment time for yohimbine administration was optimal for triggering reinstatement responding (unpublished data). In this case, oxytocin was administered after yohimbine treatment to keep the pretreatment time for the neuropeptide consistent with that used in the TMT study (Study 1). Each mouse received both yohimbine doses in a counterbalanced order with one week of extinction responding between tests. Male and female cohorts were tested separately (N= 7–10/group/sex).

Drugs

Synthetic human oxytocin (CellSciences, Canton, MA) and yohimbine (Tocris Bioscience, Minneapolis, MN), were dissolved in 0.9% saline, which served as the vehicle. Injections were administered intraperitoneally in a volume of 0.01 ml/g body weight in all experiments. Alcohol (95% ethanol) was obtained from AAPER (Shelbyville, KY) and diluted with tap water to the appropriate concentration. The alcohol solution was prepared daily and presented at room temperature.

Statistical Analysis

The primary dependent variable analyzed was lever responses during different phases of the studies. Lever responses were averaged over the last three daily sessions of baseline alcohol self-administration and the last 3 days of extinction testing prior to reinstatement testing. Number of reinforcers earned and amount of alcohol intake (g/kg) averaged over the last three self-administration sessions were also analyzed. For Study 1, lever responses, reinforcers earned, and alcohol intake in male and female mice were analyzed by t-tests.

Responses during reinstatement testing were analyzed by ANOVA, with Sex and Dose as between-subject factors. For Study 2, since male and female mice were not tested simultaneously, reinstatement responding for males and females was separately analyzed by ANOVA, with oxytocin Dose as a between-subject factor and yohimbine Treatment as a repeated measure. Initial analyses also included order of yohimbine dose as a factor but since this variable did not significantly interact with the other variables, data were presented and analyzed as collapsed over this factor. Posthoc analyses (Newman-Keuls) were conducted when appropriate. For all analyses, significance

RESULTS

Study 1: Oxytocin Effects on TMT-Induced Reinstatement of Alcohol Seeking in Male and Female Mice

All animals demonstrated stable and preferential responding on the active lever. Males and females did not significantly differ in responding on the active lever [$t(66)= 0.08$, $p> 0.1$] and inactive lever [$t(66)= 0.95$, $p> 0.1$] (Figure 1a), and there was no sex-related difference in the number of alcohol reinforcers earned [$t(66)= 0.25$, $p> 0.1$] (Figure 1b). However, average alcohol intake was significantly higher in females compared to males [$t(66)= 2.87$, $p< 0.01$] due to differences in body weight (Figure 1c).

During reinstatement testing, TMT exposure significantly increased alcohol-seeking behavior, and oxytocin treatment significantly reduced this stress-induced relapse-like responding in both male and female mice in a dose-related manner (Figure 2). ANOVA revealed a significant main effect of Dose [$F(3,63)= 8.39$, $p< 0.001$], indicating that oxytocin was effective in reducing alcohol responding in both males and females. While it appears that females may have been more sensitive to the effects of oxytocin, this was not supported by the analysis, as the Dose x Sex interaction did not achieve statistical significance.

Separate analysis of data from males revealed a significant effect of Dose [$F(3,35)= 5.22$, $p< 0.01$] and post-hoc comparisons indicated that the highest oxytocin dose tested (1 mg/kg) reduced responding compared to vehicle ($p< 0.05$). In females, a main effect of Dose [$F(3,34)= 4.54$, $p< 0.01$] and post-hoc analysis indicated that both 0.5 and 1 mg/kg doses of oxytocin significantly reduced alcohol seeking behavior compared to vehicle ($ps< 0.05$).

Study 2A: Oxytocin Effects on Yohimbine-Induced Reinstatement of Alcohol Seeking in Male Mice

Active and inactive lever responses, number of alcohol reinforcers earned, and alcohol intake over the last 3 days of oral alcohol self-administration in males are shown in Figure 3a, 3b, and 3c, respectively. Yohimbine treatment increased alcohol responding during reinstatement testing and this effect was blocked by pretreatment with oxytocin (1 mg/kg) (Figure 3d). Increased reinstatement responding following injection of yohimbine did not significantly differ as a function of dose of the drug. ANOVA revealed a significant main effect of oxytocin Dose [$F(1,17)= 22.15$, $p< 0.001$], but the yohimbine Treatment x oxytocin Dose interaction was not significant. This indicates that systemic administration of oxytocin (1

mg/kg) reduced alcohol seeking behavior provoked by both yohimbine doses to a similar extent.

Study 2B: Oxytocin Effects on Yohimbine-Induced Reinstatement of Alcohol Seeking in Female Mice

Active and inactive lever responses, number of alcohol reinforcers earned, and alcohol intake over the last 3 days of oral alcohol self-administration in females are shown in Figure 4a, 4b, and 4c, respectively. As in males, yohimbine treatment increased alcohol responding during reinstatement testing and this effect was blocked by pretreatment with oxytocin (1 mg/kg) (Figure 4d). ANOVA indicated that both doses of yohimbine produced a similar increase in alcohol responding [$F(1,26)= 1.62$, $p> 0.1$], and oxytocin blocked this increase in responding [$F(1,26)= 23.52$, $p< 0.001$]. The lack of a significant yohimbine Treatment x oxytocin Dose interaction indicates that oxytocin was effective in reducing elevated alcohol seeking produced by both doses of yohimbine.

DISCUSSION

Results from the present study demonstrate that systemic administration of oxytocin reduces stress-induced reinstatement of alcohol-seeking behavior. In Study 1, oxytocin attenuated predator odor (TMT)-induced reinstatement in a dose-related manner in both male and female mice. In Study 2, oxytocin attenuated increased alcohol-seeking following administration of the pharmacological stressor yohimbine in both sexes. Although oxytocin has been shown to effectively reduce cue- and drug-primed reinstatement to alcohol and other drugs of abuse (Baracz et al. 2015; Baracz et al. 2016; Cox et al. 2017; Ferland et al. 2016; Hansson et al. 2018; Kohtz et al. 2018; Weber et al. 2018), to our knowledge, this is the first report to demonstrate that oxytocin attenuates stress-induced alcohol relapse-like behavior in mice.

Our finding that oxytocin reduces stress-induced alcohol reinstatement responding is consistent with other studies that have shown OXT decreases stress-related relapse-like behavior to other drugs of abuse. Systemic administration of OXT decreased methamphetamine-seeking behavior following predator odor exposure (Ferland et al. 2016) and yohimbine administration (Cox et al. 2013) in rats. In mice, centrally administered OXT (icv.) attenuated reinstatement of methamphetamine conditioned place preference (CPP) induced by restraint stress (Qi et al. 2009), and systemic administration of the oxytocin analog, carbetocin, reduced the effects of forced-swim stress on reinstatement of morphine-induced CPP (Zanos et al. 2014). Collectively, results from the present study and related reports in the literature indicate that oxytocin treatment is effective in attenuating relapse-like behavior provoked by various stress procedures.

The present study demonstrated oxytocin was effective in reducing the ability of two different stress procedures (predator odor (TMT) exposure and systemic administration of the alpha-2 noradrenergic antagonist yohimbine) to reinstate alcohol-seeking behavior. While both stressors produced a significant increase in alcohol-seeking behavior, the use of TMT and yohimbine as tools to induce stress and fear responses are controversial (Blanchard et al. 2003; Chen et al. 2015; Endres and Fendt 2007; Fendt and Endres 2008;

Mantsch et al. 2016; McGregor et al. 2002). In rodents, TMT exposure reliably produces an elevation in plasma corticosterone levels (Morrow et al. 2000), and elicits a number of autonomic and behavioral changes that are indicative of fear and anxiety (Endres et al. 2005; Fendt et al. 2005). However, it has been suggested that TMT is not a fear-inducing stimulus but rather a generalized noxious odor (Fendt et al. 2005; McGregor et al. 2002). Studies investigating the neural basis of TMT effects on rodents demonstrate activation of the HPA-axis (Day et al. 2004; Myers and Rinaman 2005) and increases in *c-fos* expression in brain regions shown to be important in fear processing and stress-related responses, such as the amygdala, bed nucleus of the stria terminalis, lateral septum, and hypothalamus (Day et al. 2004; Janitzky et al. 2015). Thus, there is ample neural, physiological, and behavioral evidence indicating that TMT exposure produces a stress-like state that may trigger alcohol-seeking behavior in the operant conditioning reinstatement model.

The use of yohimbine as a stressor in this relapse model also has been called into question (Chen et al. 2015). It is suggested that yohimbine may not be exerting its effects on alcohol/drug seeking behavior by producing an aversive (stress) state but, rather, merely potentiating cue-related responding. Further, yohimbine was not found to induce conditioned place aversion, suggesting a lack of stress-like effects (Chen et al. 2015). However, evidence from several studies indicate that yohimbine engages critical components of stress circuitry that are known to mediate stress responses, including reinstatement of alcohol/drug seeking behavior (Buffalari and See 2011; Le et al. 2013; Shaham et al. 2003; Shalev et al. 2010). Further, human studies indicate that yohimbine induces both physiological and psychological stress-like responses (Greenwald et al. 2013) and alcohol craving (Umhau et al. 2011). Taken together, there is sufficient evidence indicating that both stress procedures employed in the present study produced an aversive state, and oxytocin was effective in attenuating their ability to provoke alcohol relapse-like behavior in male and female mice.

While results from the present study demonstrated that oxytocin is effective in attenuating stress-induced alcohol relapse-like behavior in both male and female mice, it is difficult to conclude whether a sex-related difference in sensitivity to this effect exists. Results from Study 1 suggest that females may exhibit greater sensitivity to OXT than males (shift to the left in the dose-effect function). Unfortunately, only a single dose was used in Study 2 and this dose (1 mg/kg OXT) was equally effective in reducing stress-induced reinstatement of alcohol seeking in both females and males. Other studies have provided evidence for sex-related differences in sensitivity to OXT. For example, lower doses of OXT were more effective in reducing cue-induced reinstatement of cocaine (Kohtz et al. 2018) and sucrose (Zhou et al. 2015) responding in female rats compared to their male counterparts. Further, sexually dimorphic expression of OXT receptors in the brain has been reported in several strains of rats and mice (Dumais et al. 2016; Dumais et al. 2013; Dumais and Veenema 2016), although no such sex differences were found in a study with C57BL/6J mice (Hammock and Levitt 2013). Oxytocin expression and signaling in the brain has been shown to be modulated by gonadal hormones (Dumais and Veenema 2016; Ivell and Walther 1999; Patchev et al. 1993). Fluctuations in estrogen and progesterone levels across the estrous cycle may influence sensitivity to OXT in females (Dumais et al. 2013; Dumais and Veenema 2016). Although we did not assess estrus status in our studies, other reports have indicated that the effects of OXT on relapse-like behavior are not dependent on phase of

estrus at the time of testing (Cox et al. 2013; Leong et al. 2017; Leong et al. 2016). Thus, possible sex-related differences in sensitivity to OXT as well as the role of sex hormones influencing effects of OXT on alcohol relapse-like behavior remain to be determined.

The mechanism by which OXT decreased stress-induced re-invigoration of alcohol responding is not well understood. Previous work in our laboratory demonstrated that oxytocin doses less than 3 mg/kg reduced alcohol self-administration in C57BL/6J mice without altering general locomotor activity (King et al., 2017). Thus, it is unlikely that oxytocin reduced alcohol relapse responding provoked by TMT or yohimbine exposure due to a nonspecific sedative effect.

Oxytocin is known to exert anti-stress and anxiolytic effects (Jurek et al. 2015; Peters et al. 2014; Slattery and Neumann 2010). Oxytocin interacts with corticotrophin-releasing hormone (CRF) neurons and the peptide has been shown to dampen stress-induced HPA-axis activation (elevated corticosterone levels), as well as reduce stress-related behavioral responses in animal models of anxiety and depression (Neumann et al. 2000; Windle et al. 2004). Further, high levels of OXT and OXT receptor mRNA expression are localized in forebrain regions such as the extended amygdala, where OXT signaling can play a significant role in the regulation of anxiety, stress, and reward-related behaviors (Dabrowska et al. 2011; Gimpl and Fahrenholz 2001; Martinon and Dabrowska 2018; Veinante and Freund-Mercier 1997).

Another potential mechanism by which oxytocin decreases reinstatement of alcohol-seeking behavior may be through direct influence on reward signaling. Oxytocin receptors have been identified in a number of brain regions known to significantly contribute to drug-related reward and relapse-like behavior. This includes the nucleus accumbens core (NAcc) (Tan et al., 2017; Dolen et al., 2013), ventral tegmental area (VTA) (Peris et al., 2017) and medial prefrontal cortex (mPFC) (Li et al, 2016; Nakajima et al., 2014). Intra-NAcc infusion of oxytocin has been shown to attenuate drug primed- (Baracz et al. 2015; Baracz et al. 2016) and cue- (Bernheim et al., 2017) induced reinstatement of methampethamine seeking. Additionally, Leong et al. (2017) demonstrated that elevated Fos expression in the mPFC, NAcc, and subthalamic nucleus (STN) in response to cocaine associated cues was normalized by a systemic injection of oxytocin in rats. Interestingly, direct infusion of oxytocin into the NAcc and STN reduced whereas intra-PFC oxytocin increased cue-induced reinstatement of cocaine-seeking behavior (Weber et al., 2018; Leong et al., 2018). A few studies have examined the influence of oxytocin on alcohol-related reward effects. For example, acute intracerebroventricular infusion of oxytocin reduced alcohol consumption and elevated dopamine release in the NAc in rats (Peters et al., 2017). Viral-mediated overexpression of oxytocin receptors in the NAc was shown to reduce alcohol drinking and alcohol-induced conditioned place preference (Bahi, 2015; Bahi et al., 2016). Hansson et al. (2018) demonstrated increases in oxytocin receptor mRNA and protein levels in prefrontal cortex, striatal, amygdala, and hippocampal regions in alcohol dependent rats after 3 weeks of abstinence, implicating similar brain regions as the aforementioned studies. Thus, there is evidence to suggest that the effects of oxytocin within brain reward circuitry may not only contribute to its ability to reduce alcohol self-administration, but also reduce alcohol relapse-like behavior. Unfortunately, there are no studies investigating the effects of

site-specific administration of oxytocin on reinstatement of alcohol-seeking behavior using operant conditioning procedures. Future studies are needed to investigate specific brain regional changes that mediate the ability of oxytocin to blunt alcohol relapse-like behavior.

Systemic administration of OXT raises the issue of blood-brain barrier penetrance (Lee et al. 2018). A number of studies have recapitulated effects of systemic OXT administration with direct (icv. or site-specific) intracranial infusions across a number of behavioral tasks (Cox et al. 2017; Lee et al. 2018; Love 2014; Ring et al. 2006; Slattery and Neumann 2010; Windle et al. 1997). Additionally, peripheral administration of OXT has been shown to induce Fos expression in OXT neurons in the PVN (Carson et al. 2010; Leong et al. 2017), suggesting that peripheral administration may induce endogenous central release. However, Lee et al. (2018) demonstrated that while OXT administered through intranasal and intravenous routes of administration increased OXT levels in cerebral spinal fluid, it did not activate a feed forward mechanism to elevate endogenous oxytocin. Thus, the mechanism by which exogenous OXT delivered in the periphery activates OXT signaling in the brain remains unclear. Further studies are needed to address this issue, as it is relevant to the potential for oxytocin to serve as a therapeutic for alcohol/drug addiction.

In conclusion, our findings reveal that systemic oxytocin administration attenuates stress-induced reinstatement of alcohol seeking in male and female mice. Exogenous OXT decreased alcohol-seeking behavior in a dose-related manner in response to acute challenge with a predator odor. The reduction in reinstatement responding was similar between males and females, though females showed a decrease in responding at lower doses of OXT compared to males. Additionally, OXT administration produced a similar decrease in alcohol relapse-like behavior triggered by the pharmacological stressor yohimbine in both sexes. Taken together, these results indicate that OXT may be potential therapeutic target for mitigating relapse initiated by stress in both males and females.

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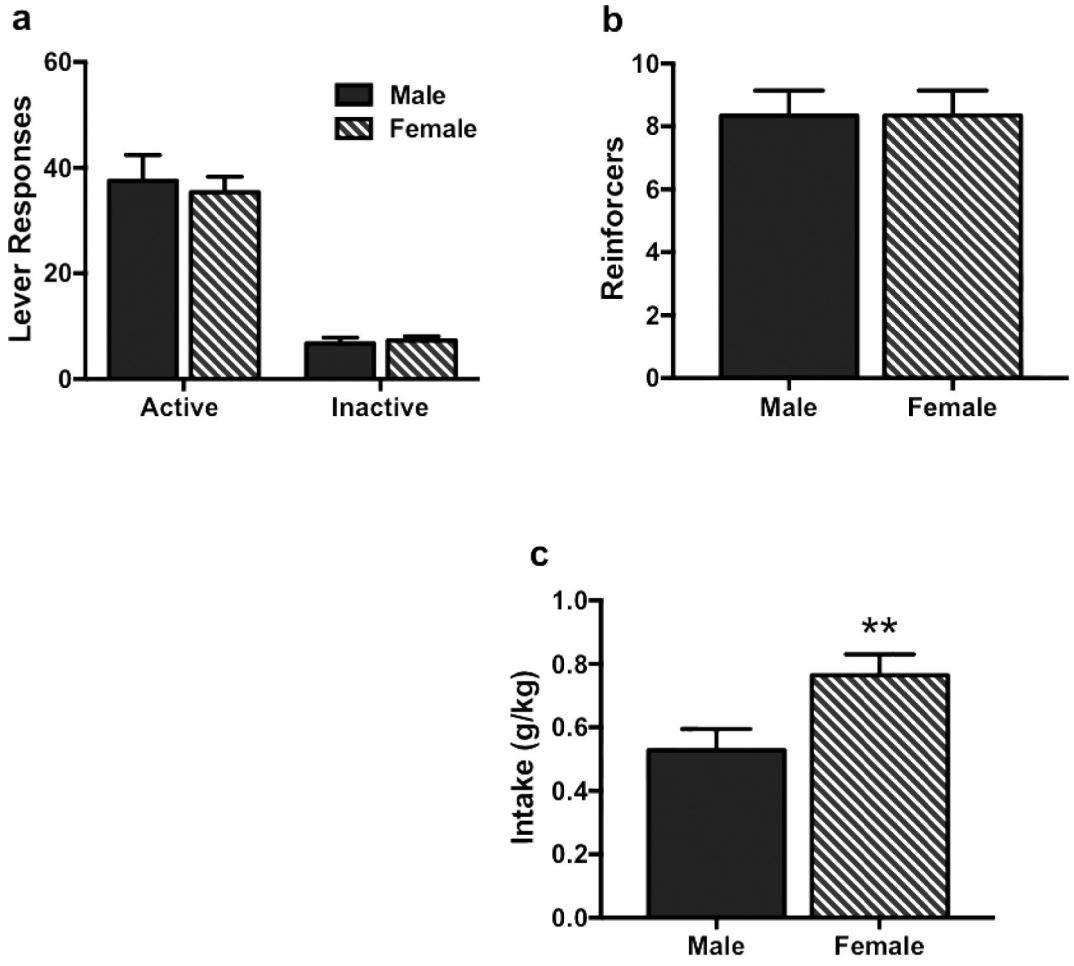

Figure 1:

Lever responding, alcohol reinforcers and alcohol Intake during oral selfadministration of alcohol using operant conditioning procedures. Males and females did not significantly differ in responding on the active lever **(a),** and there was no sex-related difference in the number of alcohol reinforcers **(b).** Average alcohol intake was significantly higher in females compared to males **(c).** Values are mean ± s.e.m. (N= 8–10/group). Significantly differs compared to males: ** ($p < 0.01$)

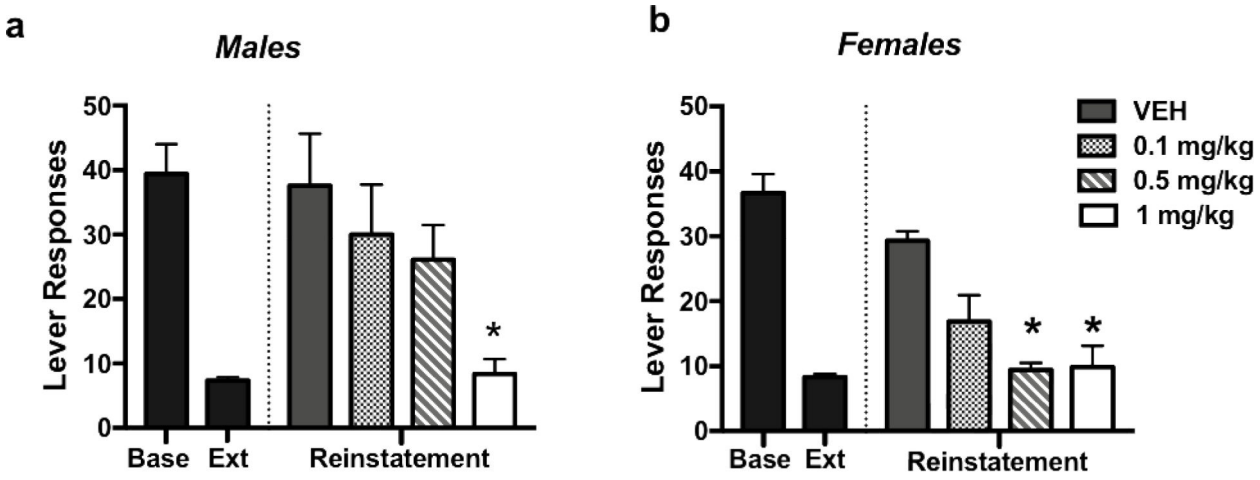

Figure 2:

Effects of oxytocin on TMT-induced reinstatement of alcohol-seeking behavior. Systemic OXT attenuates TMT-induced reinstatement of alcohol seeking behavior in a dose-related manner in male (left) and female (right) mice. Values are mean ± s.e.m. (N= 10/group). Significantly differs from vehicle condition: * ($p < 0.05$)

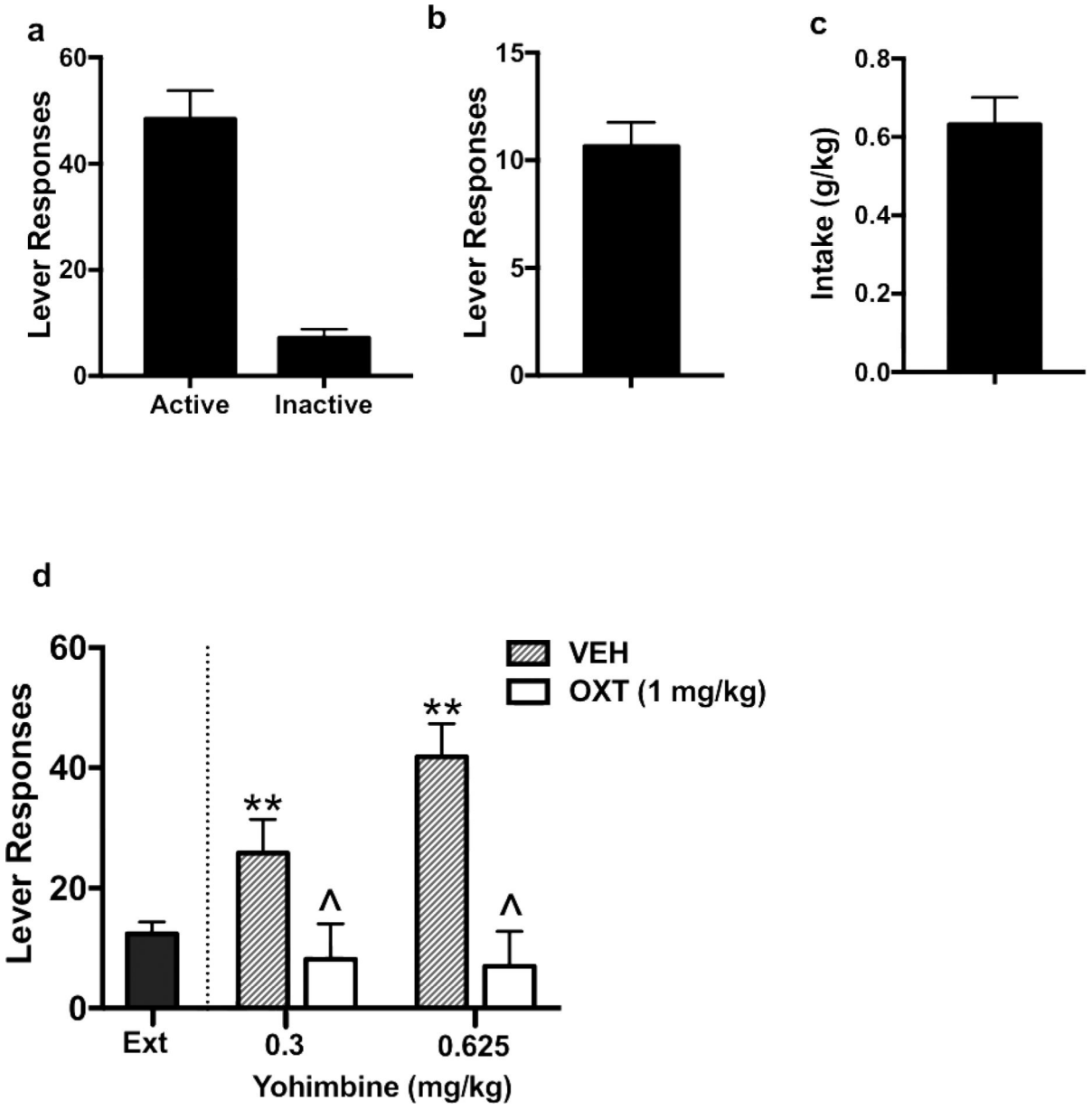

Figure 3:

Effects of oxytocin on yohimbine-induced reinstatement in male mice. Active and inactive lever responses **(a),** number of alcohol reinforcers earned **(b),** and alcohol intake **(c)** over the last 3 days of oral alcohol self-administration. Yohimbine treatment increased alcohol responding during reinstatement testing and this effect was blocked by pretreatment with OXT (1 mg/kg) **(D).** Values are mean ± s.e.m. (N= 7–10/group). Significantly differs from average last 3 days of extinction (ext): ** ($p < 0.01$). Significantly differs from vehicle condition: ^ ($p < 0.05$).

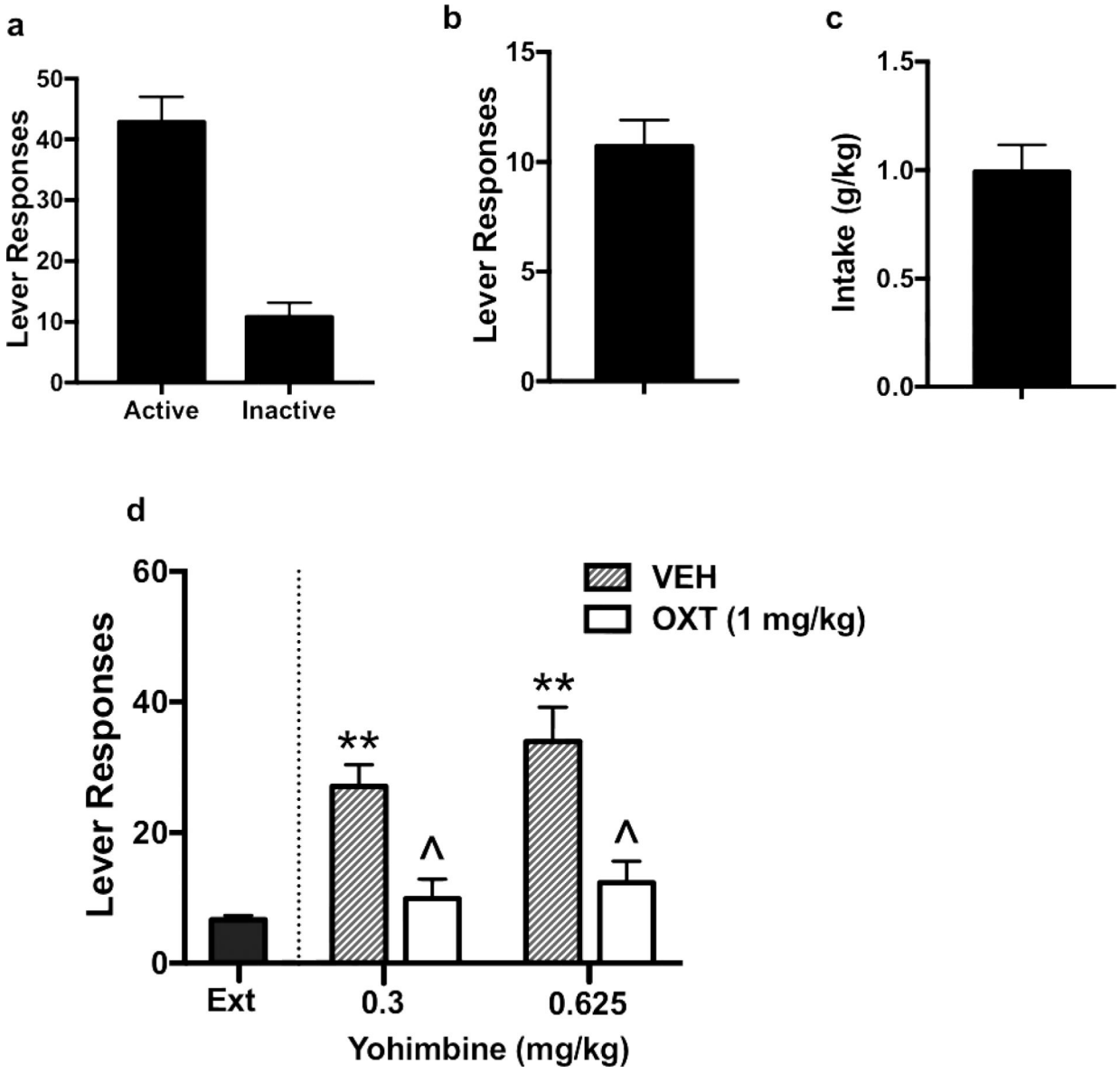

Figure 4:

Effects of oxytocin on yohimbine-induced reinstatement in female mice. Active and inactive lever responses **(a),** number of alcohol reinforcers earned **(b),** and alcohol intake **(c)** over the last 3 days of oral alcohol self-administration. Similar to males, yohimbine treatment increased alcohol responding during reinstatement testing and this effect was attenuated by pretreatment with OXT (1 mg/kg) **(d).** Values are mean ± s.e.m. (N= 7–10/group). Significantly differs from average last 3 days of extinction (ext): ** ($p < 0.01$). Significantly differs from vehicle condition: ^ ($p < 0.05$).