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Effects of oxytocin administration on fear-potentiated acoustic startle in co-occurring PTSD and alcohol use disorder: A randomized clinical trial

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Abstract

Co-occurring posttraumatic stress disorder (PTSD) and alcohol use disorder (AUD) is common and particularly associated with elevation of hyperarousal compared to PTSD alone. Treatment options are limited. Oxytocin regulates physiological stress response. Intranasal oxytocin administration has demonstrated potential in reducing symptoms of both PTSD and AUD. This study addresses a gap in the literature by investigating effects of intranasal oxytocin on startle reactivity, an important potential marker of both PTSD and AUD symptomatology. This is a randomized, double-blind, placebo-controlled, within- and between-participant, crossover, dose-ranging study examining the effects of a single administration of oxytocin 20 IU versus 40 IU versus placebo on psychophysiological responses to a common laboratory fear-potentiated acoustic startle paradigm in participants with PTSD-AUD (n = 47) and controls (n = 37) under three different levels of threat. Contrary to our hypothesis, for the PTSD-AUD group, oxytocin 20 IU had no effect on startle reactivity, while oxytocin 40 IU increased measures of startle reactivity.

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Declaration of Competing Interest
No potential conflicts of interest are declared by the authors.

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Christopher S. Stauffer: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Project administration, Funding acquisition, Writing – review & editing. **Tyler E. Morrison:** Validation, Data curation. **Nathan K. Meinzer:** Formal analysis. **David Leung:** Software, Investigation, Data curation, Writing – review & editing. **Jessica Buffington:** Investigation, Writing – review & editing. **Evan G. Sheh:** Investigation, Data curation, Writing – review & editing. **Thomas C. Neylan:** Supervision, Writing – review & editing. **Aoife O'Donovan:** Resources, Writing – review & editing. **Joshua D. Woolley:** Conceptualization, Methodology, Supervision, Funding acquisition, Writing – review & editing.

Ethical Standards
The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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Additionally, for PTSD-AUD only, ambiguous versus low threat was associated with an elevated skin conductance response. For controls only, oxytocin 20 IU versus placebo was associated with reduced startle reactivity.

Keywords

Stress disorders; Post-traumatic; Alcoholism; Comorbidity; Oxytocin; Reflex; Startle; Psychophysiology

1. Introduction

Lifetime prevalence of posttraumatic stress disorder (PTSD) is estimated to be 6.8% for a general sample of Americans (Kessler et al., 2005) and 13.8% among a sample of military Veterans previously deployed to Iraq and Afghanistan (Gradus, 2019; Tanielian et al., 2008). Alcohol use disorder (AUD) is among the most commonly co-occurring disorders with PTSD (Kessler et al., 1995; McFarlane, 1998; Mills et al., 2006). Among individuals with PTSD, the prevalence of comorbid alcohol misuse has been found to range from 9.8% to as high as 61.3% (Debell et al., 2014). PTSD and AUD are both chronic and relapsing conditions with shared vulnerability factors (McLeod et al., 2001) and common neurobiological dysfunction in various brain regions, including the amygdala, hippocampus, and ventromedial prefrontal cortex (Gilpin and Weiner, 2017). Both disorders are also associated with dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, which plays a fundamental role in responding to stressful stimuli (Donadon et al., 2018; Gilpin and Weiner, 2017; Norman et al., 2012). Among individuals with a dual diagnosis of PTSD and AUD, quality of life and overall prognosis are worse than for individuals with either disorder alone—including an increased risk of suicidality and higher mortality rates (Blanco et al., 2013; Heinz et al., 2016). Moreover, the PTSD symptom cluster of hyperarousal is particularly elevated in individuals with comorbid PTSD and AUD compared to individuals with PTSD alone (Debell et al., 2014; Saladin et al., 1995). Recent trials have begun to explore treatment options for individuals with this dual diagnosis (Petrakis and Simpson, 2017; Taylor et al., 2017); however, most individual treatments do not target both PTSD and AUD symptomatology. Thus far, there is no current consensus on an optimal treatment approach for co-occurring PTSD and AUD. In sum, PTSD and AUD occur frequently together and involve overlapping neurobiology and mutual symptom exacerbation, and current treatment options are limited. Innovative interventions targeting the unique symptom profile of this dual diagnosis are gravely needed.

Oxytocin, a hypothalamic neuropeptide, serves an allostatic function in response to stress via its role in HPA axis regulation (Cardoso et al., 2014; Donadon et al., 2018; King et al., 2020; Neumann et al., 2000; Quintana and Guastella, 2020). Adverse childhood events can negatively impact development of the oxytocinergic system, leading to increased susceptibility to stress and risk of developing PTSD and substance use disorders (Johnson and Buisman-Pijlman, 2016). Knowledge of oxytocin's role in learning and memory, and its potential to ameliorate maladaptive learning such as that seen in PTSD and AUD, has inspired decades of preclinical research into how oxytocin might facilitate fear conditioning

and extinction (Cohen et al., 2010; Hou et al., 2015; Ibragimov, 1989; Martinon et al., 2019; Wang et al., 2019) and reduce addiction-related behaviors (King et al., 2020; Kovács et al., 1985; 1985; Lee et al., 2016). More recently, clinical trialists have begun to investigate intranasal oxytocin administration for the treatment of PTSD (Donadon et al., 2018; Frijling, 2017; Giovanna et al., 2020;Koch et al., 2014, 2016) and substance use disorders (Lee and Weerts, 2016; Mitchell et al., 2016; Stauffer et al., 2016; Stauffer et al., 2019; Woolley et al., 2016). Existing evidence demonstrates that intranasal oxytocin could be a safe pharmacological intervention for both PTSD and substance use disorders; however, results are mixed, and more evidence is needed in this area of research.

To date, there are only a handful of reports on the effects of intranasal oxytocin administered to individuals with a dual diagnosis of PTSD and AUD. One recent study examining the effects of intranasal oxytocin (40IU) compared to placebo in individuals with comorbid PTSD and AUD on stress reactivity during a Trier Social Stress Test found that oxytocin administration was associated with reduced cortisol levels (Flanagan et al., 2019). Both our group (Stauffer et al., 2019) and Flanagan et al. (2019) reported that a single administration of oxytocin (40IU) had no acute effect on alcohol craving in individuals with comorbid PTSD and AUD. We also replicated a finding that oxytocin increased automatic imitation of hand movements in controls, but did not observe this effect in individuals with comorbid PTSD-AUD (Morrison et al., 2020). Data are inconclusive, and the field continues to investigate if and how oxytocin administration might be helpful in the treatment of comorbid PTSD and AUD.

The startle response has commonly been used to study hyperarousal and fear in the laboratory setting (Davis, 1990; Grillon and Davis, 1997; Jovanovic et al., 2006; LaBar et al., 1995). In response to a sudden unexpected stimulus, the amygdala sends signals to the brainstem and hypothalamus, eliciting an eyeblink response measurable via electromyography (EMG) and autonomic changes in skin conductance and heart rate (Davis et al., 2010; Koch, 1999). Individuals with PTSD, and especially those with co-occurring AUD, have exhibited a hyperactive fear-potentiated startle response in the laboratory (Butler et al., 1990; Gorka, 2020; Grillon et al., 1998; Morgan et al., 1996; Niles et al., 2018; Orr et al., 1997, 1997; Pedersen et al., 2013; Shalev et al., 1997). Animal models of the fear-potentiated acoustic startle paradigm have demonstrated evidence for therapeutic effects of oxytocin administration (Ayers et al., 2011; Missig et al., 2010). In humans, oxytocin receptor genotype has been shown to affect startle response in new mothers (Comasco et al., 2016). Among healthy individuals, a single administration of oxytocin dampened startle response in one study (Ellenbogen et al., 2014) and enhanced startle response in the setting of aversive social information in another study. According to the social salience hypothesis of oxytocin (Shamay-Tsoory, 2016), oxytocin regulates attention to external social cues regardless of the valence. Although this salience effect is also dependent on baseline characteristics (such as degree of psychopathology). No studies to date have investigated the effects of intranasal oxytocin on the fear-potentiated startle response in individuals with PTSD or AUD, who are known to have heightened startle reactivity compared to healthy controls.

The current study tested the effects of a single administration of intranasal oxytocin on fear-potentiated startle reactivity in an otherwise neutral social context among individuals with comorbid PTSD-AUD and controls without PTSD or AUD. Dose-response information for intranasal oxytocin for any indication or clinical population in human trials is generally lacking (Wynn et al., 2019), which confounds the field's ability to adequately interpret complex findings in existing trials. Therefore, we employed a dose-ranging study design. Psychological research in general, and oxytocin research in particular, has suffered from low statistical power and failure to replicate. Therefore, we used a within-participant design to increase analytic power and preregistered our aims and analytic approach (see NCT02469259 and https://a-spredicted.org/zx6za.pdf - #8732). We hypothesized that individuals with PTSD-AUD would demonstrate stronger physiological responsiveness to fear-potentiated acoustic startle stimuli compared to controls and that both groups would demonstrate reduced responsiveness after receiving intranasal oxytocin versus placebo.

2. Methods

2.1. Trial design

This is a randomized, double-blind, placebo-controlled, within- and between-participant, crossover, dose-ranging study of the effects of a single administration of oxytocin on response to a fear-potentiated acoustic startle paradigm in individuals with comorbid PTSD and AUD (PTSD-AUD) and age-matched and education-matched controls. Our dose-ranging design examined the efficacy of three different treatment conditions—placebo, oxytocin 20 International Units (IU), and oxytocin 40 IU—administered in a randomized order across three experimental sessions at least one week apart. At each experimental session, we utilized a fear-potentiated startle paradigm with three different levels of threat: low, ambiguous, and high.

The University of California, San Francisco (UCSF) Institutional Review Board provided ethics review and safety monitoring for this study. All study procedures took place at the San Francisco Veterans Affairs Medical Center. Participants were compensated for each completed study visit.

2.2. Participants

Participants were recruited using mental health clinician referrals and advertisements from the San Francisco Veterans Affairs Health Care System, community mental health programs throughout San Francisco, and Craigslist. A brief, structured telephone interview was used to screen for preliminary eligibility. All participants were 18 to 75 years old. Those in the PTSD-AUD group met criteria for current PTSD and AUD in the past 12 months, as defined by the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (*DSM-5*).

Excluded from this study were individuals with: dementia or other neuropsychiatric disorders impairing ability to complete study-related tasks, clinically significant unstable medical conditions, moderate-severe acute alcohol withdrawal, current participation in a legally mandated alcohol treatment program, suicidal ideation with intent or plan in the past 30 days, suicide attempt in the past 6 months, a seizure disorder requiring

anticonvulsant medication, sensitivity to preservatives used in study drug nasal spray (i.e., potassium sorbate or mannitol), pathology of the nasal parenchyma preventing intranasal drug administration or absorption, or hormone supplementation (which may affect the endogenous oxytocin system).

Controls could not have a history of PTSD (full or partial), moderate to severe AUD, or a substance use disorder as determined by DSM-5 criteria.

2.3. Intervention

2.3.1. Study drug—Study drug consisted of oxytocin 20 IU, oxytocin 40 IU, and matched placebo and was provided by Wellspring Compounding Pharmacy (now called Valor Compounding Pharmacy, Berkeley, CA). Trained staff administered the study drug intranasally according to standardized techniques (Guastella et al., 2013) using a mucosal atomization device (MAD300; Teleflex, Morrisville, NC) affixed to a Luer lock syringe. A research pharmacist loaded the appropriate study drug and concentration into two 1-milliliter (mL) syringes. The first syringe contained 0.56 mL to accommodate the 0.06 mL dead space in the MAD300. The second syringe contained 0.50 mL and was administered into the opposite nostril. Previous work has shown that intranasal oxytocin administration begins to have a physiological effect (i.e. increase in oxytocin blood levels; decreased arousal ratings to human threat stimuli) within 30 min and lasts for at least 90 min (Gossen et al., 2012; Striepens et al., 2013). Therefore, immediately following study drug administration and prior to any study-related tasks, participants watched one of three thirty-minute neutral videos (e.g., documentary about a historical event) presented in a randomized order across experimental sessions.

2.3.2. Randomization and blinding—Our randomization procedure assigned all three treatment conditions to each participant across the three experimental sessions, respectively, in a randomized order. Participants with PTSD-AUD and controls were treatment-order-randomized separately, and each randomization plan used balanced permutations in blocks of twenty, assuming 120 participants. A research pharmacist maintained the blind and oversaw randomization using the second generator from www.randomization.com (reproduction can be viewed using seeds 4243 and 564 for PTSD-AUD and 19,293 for controls). Study staff remained blinded to study drug until data collection was complete for all participants.

2.4. Procedures

To determine full eligibility, participants attended an in-person screening assessment where written informed consent was obtained prior to any study procedures. During screening, participants completed baseline assessments. Trained clinical interviewers with at least Masters' level training in clinical psychology conducted clinical and diagnostic interviews, including the Structured Clinical Interview for *DSM-5* (SCID-5), Research Version (First et al., 2015) and the Clinician-Administered PTSD Scale for *DSM-5* (CAPS-5) (Weathers et al., 2013). An examination of the nasal parenchyma was conducted by a study physician.

Further study participation consisted of three experimental sessions each separated by at least one week. Research assistants were trained to conduct the protocol with a neutral demeanor and wore white lab coats to standardize social context. Each participant was assigned the same primary research assistant across the three experimental sessions (Chapman et al., 2018). Additional study tasks not described in this manuscript have been published separately (Morrison et al., 2020; Stauffer et al., 2019).

At all study visits, participants provided a urine sample to screen for illicit substances using a CLIA-waived, 10-panel, Discover Urine Drug Test Cup (American Screening Corp., Shreveport, LA). Participants were also screened for acute alcohol consumption using the BACtrack S80 Breathalyzer (BACtrack, San Francisco, CA). If any participant had a breath alcohol concentration of more than 0.00%, or a control participant had a positive urine drug screen other than for tetrahydrocannabinol, their visit was rescheduled for another day. Participants were asked to refrain from using nicotine products starting at the beginning of each experimental session until after study procedures.

Participants completed all self-assessment measures using Research Electronic Data Capture (RedCap), an online data capture tool hosted by the Department of Veterans Affairs, using an iPad (Apple, Cupertino, CA; iOS version 5.1.1). For timing of assessments, see Table 1.

2.4.1. Fear-Potentiated acoustic startle paradigm—Approximately 30 min after study drug administration, a fear-potentiated acoustic startle paradigm (Pole et al., 2003, 2007) was used to assess startle reactivity to simultaneous acoustic and visual stimuli potentiated by the threat of electric shock. Participants sat in a chair in front of a computer monitor and were told that they would hear brief, loud sounds through the headphones during the task. They were instructed to keep their attention focused on the fixation cross in the center of the screen in front of them and to refrain from moving their arms or legs or closing their eyes, except to blink. Acoustic startle stimuli were 40-millisecond (ms) white-noise bursts generated by a San Diego Instruments Startle Reflex System (SR-Lab, San Diego, CA, USA) and delivered at randomized intervals through noise-cancelling headphones to both ears at 115 A-weighted decibels (dBA) with 0-ms rise and fall times over 70-dBA background noise. A visual threat stimulus accompanied each acoustic startle stimulus and consisted of a small cross on a computer screen that would suddenly increase in size to fill the screen then return to original size.

At each experimental session, participants experienced three threat conditions: low, ambiguous, and high. Each condition lasted approximately four minutes and was separated by approximately one minute. First, under the *low threat condition,* each participant received ten acoustic startle stimuli with no shock cable yet in place. Responses to the first five stimuli were used only for habituation and not included in analyses. After completion of the low threat condition, the research assistant attached cables to two EL503 electrodes placed over the adductor pollicis muscle of the participant's dominant hand, technically making shocks possible. Electric shocks were 10-Volt, 5-ms, isolated pulse currents generated by a Stimulation Isolation Adapter ("STMI-SOC"; Biopac Systems, Goleta, CA, USA) and monitored by a Cedrus StimTracker (Cedrus, Los Angeles, CA, USA). The appropriate amperage was determined for each participant individually during the screening visit by

gradually increasing from 0.2 milliamps (mA) until they reported the shock to be "annoying, but not painful", with a maximum of 5 mA. Participants were told they would hear the same sounds as in the previous condition, but would only receive shocks if the words "SHOCK POSSIBLE" appeared on the computer monitor with the fixation cross. During the *ambiguous threat condition,* though shock cables were in place, the computer screen displayed the safety cue "SHOCK NOT POSSIBLE", and no shocks were delivered. During the *high threat condition*, shock cables were in place and the screen displayed "SHOCK POSSIBLE". However, no shocks were delivered during this condition either. Participants experienced ambiguous and high threat conditions in a randomized order, undergoing five acoustic/visual startle stimuli for each. The paradigm concluded with a final *shock condition*, which was identical to the high threat condition, with the screen displaying "SHOCK POSSIBLE", except that this was the only time in the experiment when participants actually received a shock. This condition was carried out to ensure participants believed shocks were possible on subsequent experimental sessions, but these trials were excluded from analyses. Participants were randomized to receive either one or two shocks (experiencing each at least once) within the first 30 to 180 s of the final shock condition.

2.5. Dependent variables from startle paradigm

2.5.1. Electromyography response (EMGR)—Electromyography (EMG) recordings were obtained using EL254 Ag/ AgCl electrodes in a three-lead configuration, where two electrodes were placed on the orbicularis oculi muscle and a third ground electrode was placed in the center of the forehead above the brow line. Prior to electrode placement, skin was abraded using electrode abrading pads (ELPAD) and sterilized with Webcol 70% isopropyl alcohol prep pads, and a 0.5% chloride-salt electrode gel (GEL 100) was applied under each electrode to enhance signal conduction. A BIOPAC Systems electrode checker (EL-CHECK) was used to ensure that impedance between Vin+ and Vin- was below 10 kiloohms. The EMG signal was amplified using EMG2-R, filtered using a finite impulse response band pass of 13 hertz (Hz) to 500 Hz, digitally transformed using a comb band stop at 60 Hz line frequency, and divided by window size. The average rectified signal was then derived using a 5-ms window. EMGR was defined as the maximum EMG voltage, in microvolts, occurring between 21 and 200 ms after a given acoustic startle stimulus subtracted from the mean EMG voltage over the 1 s period prior to the same startle stimulus (Pole et al., 2009).

2.5.2. Skin conductance response (SCR)—Electrodermal activity (EDA) was measured using EL507 Ag/AgCl electrodes in a two-lead configuration, with electrodes placed on the palm of the non-dominant hand along the flexor digiti minimi muscle. Again, chloride salt electrode gel was applied to each electrode. The skin conductance level (SCL) signal, measured in microsiemens, was amplified using EDA100C set to a gain of 10 microsiemens/Volt, a direct current high-pass filter, and a 0.05 Hz low-pass notch filter. SCR was defined as the peak SCL occurring between 1 and 4 s after a given acoustic startle stimulus subtracted from the mean SCL over the 1 s period prior to the same startle stimulus (Pole et al., 2009).

2.5.3. Heart rate response (HRR)—

Electrocardiogram (ECG) was obtained using EL503 Ag/AgCl electrodes in a three-lead configuration, where an electrode was placed underneath both the right and left clavicle and a third electrode was placed beneath the bottom rib on the left side. The signal was amplified using ECG100C set to a gain of 2000, a 1.0 Hz high-pass filter, and a 35 Hz low-pass notch filter. The ECG signal was digitally filtered for noise using a finite impulse response low-pass filter of 15 to 35 Hz, with AcqKnowledge software (version 5.0.0). R peaks were automatically identified by AcqKnowledge, then individually confirmed by trained study staff, in order to attain the heart rate (HR), beats per minute during any given time period. HRR was defined as the maximum HR occurring between 1 and 4 s after a given acoustic startle stimulus subtracted from the mean HR over the 1 s period prior to the same startle stimulus (Pole et al., 2009).

Measurements for all three psychophysiological signals were sampled at 1000 Hz using hardware from BIOPAC Systems and AcqKnowledge software (version 4.3.1). For each individual, five trials from each threat condition for a given experimental session were averaged for each psychophysiological outcome.

2.6. Statistical methods

Outliers for relevant physiological and self-report measures were identified. All outliers were defined as >3 standard deviations (SDs) from the mean with the exception of maximum post-startle stimulus values for EMG, SCL, and HR, which were defined as >4SD above the mean (Niles et al., 2018). Outlier data points were excluded from analysis.

Using a maximal approach, we explored covariate effects for each psychophysiology outcome by testing for fixed effects of oxytocin treatment along with fixed effects of all covariates: age, race, education, smoking status, Veteran status, proportion of drinking days out of past 30 days, relationship status, gender of research assistant (Chapman et al., 2018), and study drug order. Continuous variables were rescaled and centered using Gellman's rescaling function in R in order to standardize comparison of effect sizes (Gelman and Hill, 2007). Categorical variables were entered as factors. Significant covariates ($p \leq 05$) for a specific outcome were controlled for in subsequent models for that outcome.

We applied a linear mixed effects model (LMEM) to our psychophysiology outcomes separately using R (version 3.6.1.) and select packages (Bates et al., 2014; Gelman, 2018; Gelman and Hill, 2007; Kuznetsova et al., 2017; R Core Team, 2018). Placebo treatment, low threat, and control participants were used as reference groups in the various models. Observations were grouped by participant to ascertain changes within-participant and between-participant across treatment levels. LMEMs accommodate missing data, using all available data to estimate the model (Hedeker and Gibbons, 2006). We assume that random effects are drawn from a bivariate, normal distribution with mean (0,0) with free parameters random slope variance, random intercept variance, and intercept/slope covariance.

3. Results

3.1. Recruitment & participant flow

56 PTSD-AUD and 45 controls were enrolled between 5/24/2016 and 12/8/2017. We excluded from analysis the four female participants. Due to early challenges recruiting women for our study, we opted to focus subsequent recruitment efforts on male participants only because of the known variation in endogenous oxytocin system functioning and response to intranasal oxytocin in both preclinical and clinical studies based on gender. We also excluded from analysis any participants who completed only 1 study visit (PTSD-AUD = 7, controls = 6). Thus, 47 PTSD-AUD and 37 controls were included in analyses. Of note, 2 participants in the PTSD-AUD group and 2 participants in the control group completed 2 study visits, the remainder completed all 3 study visits. For a visualization of participant flow from initial recruitment through analysis, see Fig. 1.

3.2. Baseline data

See Table 2 for demographic information and baseline characteristics. Participants with PTSD-AUD were more likely to be Veterans and tobacco smokers.

3.3. Data cleaning

For EMGR, 116 out of 3780 trials [PTSD-AUD: 64 (3.03% of total PTSD-AUD trials), controls: 52 (3.12% of total control trials)] were removed because EMG values were outliers as defined above. An additional 983 trials (PTSD-AUD: 584, controls: 399) were removed because the EMG signal was not readable or the participant did not demonstrate an EMGR based on visual inspection of the signal. For SCR, 119 trials [PTSD-AUD: 71 (3.36% of total PTSD-AUD trials), controls, 48 (2.88% of total control trials)] were removed because SCL values were outliers as defined above. In addition, 1760 trials (PTSD-AUD: 1105, controls: 655) were removed because there was no interpretable data or the participant did not show a SCR of at least 0.02 μS. Prior research shows that approximately 10%–25% of participants, or up to 40–50% in some clinical groups, are “non-responders” and do not provide a reliable SCR (Ikezawa et al., 2012; Cacioppo et al., 2017, p233). For HRR, 21 trials [PTSD-AUD: 13 (0.06% of total PTSD-AUD trials), controls: 8 (0.05% of total control trials)] were removed because HR values were outliers as defined above. In addition, 30 trials (PTSD-AUD: 16, controls: 14) were removed because excessive EKG signal noise interfered with detection of R-R peaks. No participants were entirely excluded from analysis for uninterpretable data. Averaging trials from the same treatment session and threat condition for individuals, the maximum number of observations would be 756. Our analyses for EMG, SCR, and HRR included a total of 691, 638, and 748 observations, respectively.

3.4. Control variables

There was a significant effect of age on both EMGR [−19.98(9.61), $p = .041$] and SCR [B(SE) = −0.23(0.06), $p < 0.001$] such that increased age was associated with diminished startle responses. Additionally, being in a relationship [0.18(0.06), $p = .002$] and being a Veteran [0.16 (0.06), $p = .013$] were both associated with significantly increased SCR. There

were no significant effects of race, education, smoking status, proportion of drinking days out of past 30 days, gender of research assistant, or study drug order.

3.5. Outcomes

There were no significant differences in startle reactivity between the PTSD-AUD group and controls when looking at the placebo condition only (Model 1). Results from the subsequent LMEM models for the overall sample (Model 2a), PTSD-AUD only (Model 2b), and controls only (Model 2c) are outlined below by psychophysiology outcome measure.

Electromyography Response.—For EMGR, there was no significant interaction between treatment and threat condition. We observed a significant main effect of oxytocin 40 IU for the overall sample and PTSD-AUD only, and a trend-level main effect of oxytocin 40 IU for controls only, such that oxytocin 40 IU resulted in increased EMGR compared to placebo (see Table 3 and Fig. 2a). There was no significant effect of 20 IU or threat condition, regardless of group.

Skin Conductance Response.—For SCR, there was no significant interaction between treatment and threat condition. We observed a significant main effect of oxytocin 40 IU for the overall sample and for PTSD-AUD only, but not for controls only, such that oxytocin 40 IU resulted in increased SCR compared to placebo (see Table 3 and Fig. 2b). Conversely, for controls only, oxytocin 20 IU was associated with significantly lower SCR. For PTSD-AUD only, ambiguous versus low threat was associated with a significantly higher SCR irrespective of drug condition.

Heart Rate Response.—For HRR, there was no significant interaction between treatment and threat condition. We observed a trend-level main effect of oxytocin 20 IU for controls only, indicating a reduction in HRR compared to placebo (see Table 3 and Fig. 2c). There was no significant effect of 40 IU on HRR. High threat, compared to low threat, was associated with a trend-level reduction in HRR for the overall sample, but not for PTSD-AUD or controls alone.

4. Discussion

In contrast to our hypotheses, oxytocin 40 IU compared to placebo was associated with significant increases in both eye blink and skin conductance response to startle cues in the overall sample. This remained significant for the PTSD-AUD group only but not for controls (although oxytocin 40 IU was associated with a trend-level increase in EMGR for controls). Oxytocin 20 IU was associated with significantly reduced SCR, and trend-level reduction in HRR, for controls only. Thus, while our findings suggest that oxytocin 20 IU may reduce some markers of startle reactivity for controls, this did not hold true for the PTSD-AUD group. Overall, these findings highlight the importance of paying attention to clinical status and dosage in single administration oxytocin studies in general, and in particular when examining effects on the startle response.

Our findings suggest that a single administration of oxytocin does not reduce acute startle reactivity for individuals with PTSD and AUD. In fact, 40 IU was shown to increase eye

blink and skin conductance responsiveness to fear-potentiated acoustic startle stimuli. This is consistent with a recent meta-analysis reporting that a single administration of intranasal oxytocin to healthy individuals is associated with significantly increased physiological startle response to threat with a small effect size (Leppanen et al., 2018). However, in contrast, one study not included in this meta-analysis reported significantly *diminished* acoustic startle reflex after administration of oxytocin 24 IU (Ellenbogen et al., 2014). Despite mixed reports in the literature, our findings introduce a clinical sample into the existing oxytocin-startle literature for the first time.

Donadon et al. (2018) conclude in their oxytocin and trauma review that oxytocin administration restored function in neural networks associated with fear control and extinction in individuals with PTSD. Depending on how you look at it, our main outcome either contrasts with or supports this conclusion. A contrasting interpretation may see increased startle as evidence of acute stress, which can impair extinction learning (Raio et al., 2014), and conclude that oxytocin 40 IU would not be helpful for individuals with PTSD-AUD. An alternative interpretation may conclude that the increased startle response observed in the present study is an indication of therapeutic potential rather than a sign of clinical worsening. A principal foundation of prolonged exposure therapy, a gold-standard treatment for PTSD (Rauch et al., 2012), is that imaginal trauma ‘exposures’ in a safe context must be paired with physiological activation for extinction learning to occur, whereas lower activation during first exposure is associated with later treatment drop-out (Norton et al., 2011). This is in line with the hypothesized mechanisms of facilitated fear extinction plus memory reconsolidation from pairing yohimbine (Tuerk et al., 2018) or MDMA (Feduccia and Mithoefer, 2018) with psychotherapy for the treatment of PTSD by increasing the ‘window of tolerance’ for retrieving and processing, rather than repressing, fear. Interestingly, results from randomized controlled clinical trials of individuals with PTSD have begun to demonstrate that oxytocin administration, when paired with prolonged exposure therapy, may reduce PTSD as well as depression symptoms (Flanagan et al., 2018, 2019). With mixed results, the social salience hypothesis of oxytocin (Shamay-Tsoory, 2016) highlights the importance of controlling for context in future oxytocin research (e.g., prolonged exposure with a supportive psychotherapist versus a stress-inducing laboratory task).

For the PTSD-AUD group only, across all treatment conditions, the ambiguous versus low threat condition was associated with a significantly greater SCR, but there were no significant differences in startle response between threat conditions in the control sample. Similarly, Niles et al. (2018) found that startle was particularly elevated under an ambiguous threat condition (i.e., “when safety signals are available but a possibility of danger remains”) for individuals with PTSD. However, while Niles et al. (2018) showed higher startle response among individuals with PTSD compared to those without PTSD, we did not find any significant differences between startle reactivity in our PTSD-AUD and control groups after receiving inactive placebo, even for the ambiguous condition. It is unclear if this discrepancy from prior research is due to differences in study population (e.g., comorbid AUD in addition to PTSD) or other subtle differences in study design (e.g., methodology for determining amperage of shock). This calls into question whether the behavioral testing

condition differentially affected those with PTSD-AUD versus controls, which challenges the interpretation of effects. Larger samples may help elucidate.

This study has several limitations. While studies selecting individuals with both AUD and PTSD are important given the high comorbidity of these disorders, this limits the generalizability of our results to those without one or both of these disorders. Future studies should include all genders given gender differences in the endogenous oxytocin system and in dose-responsiveness (Gao et al., 2016; Hoge et al., 2014). Future studies of oxytocin's role in the treatment of PTSD and addiction should also take care to include sexual and gender minorities, including non-binary genders, given the higher prevalence of PTSD and substance use disorders among this population and historical exclusion from PTSD research (Livingston et al., 2020; Shipherd et al., 2019). Previous studies in healthy and other clinical populations have noted additional individual moderating factors affecting responsiveness to oxytocin administration, including childhood adverse experiences, attachment anxiety, and attachment avoidance (MacDonald and Feifel, 2014; MacDonald, 2013). Our statistical power is limited due to our small sample size, although we did implement a within-subject design and pre-registered our aims and analytic approach in an attempt to mitigate this common limitation. Nonetheless, results will need to be confirmed through replication. We suggest that future studies continue to implement tiered dosing designs until dose-response curves are more well-defined.

In conclusion, a single 40 IU administration of intranasal oxytocin to participants with co-morbid PTSD and AUD was associated with a heightened acoustic startle response, as measured by evoked eye blink and skin conductance, regardless of the threat condition. Oxytocin 20 IU was associated with reduced startle reactivity, as measured by skin conductance and heart rate, for controls only. Lastly, the PTSD-AUD group had a greater startle reaction, as measured by skin conductance, when under ambiguous threat compared to low threat. Overall, research on mechanisms of action and how to best incorporate oxytocin administration into clinical treatment for PTSD and AUD is in the early stages and requires replication and further exploration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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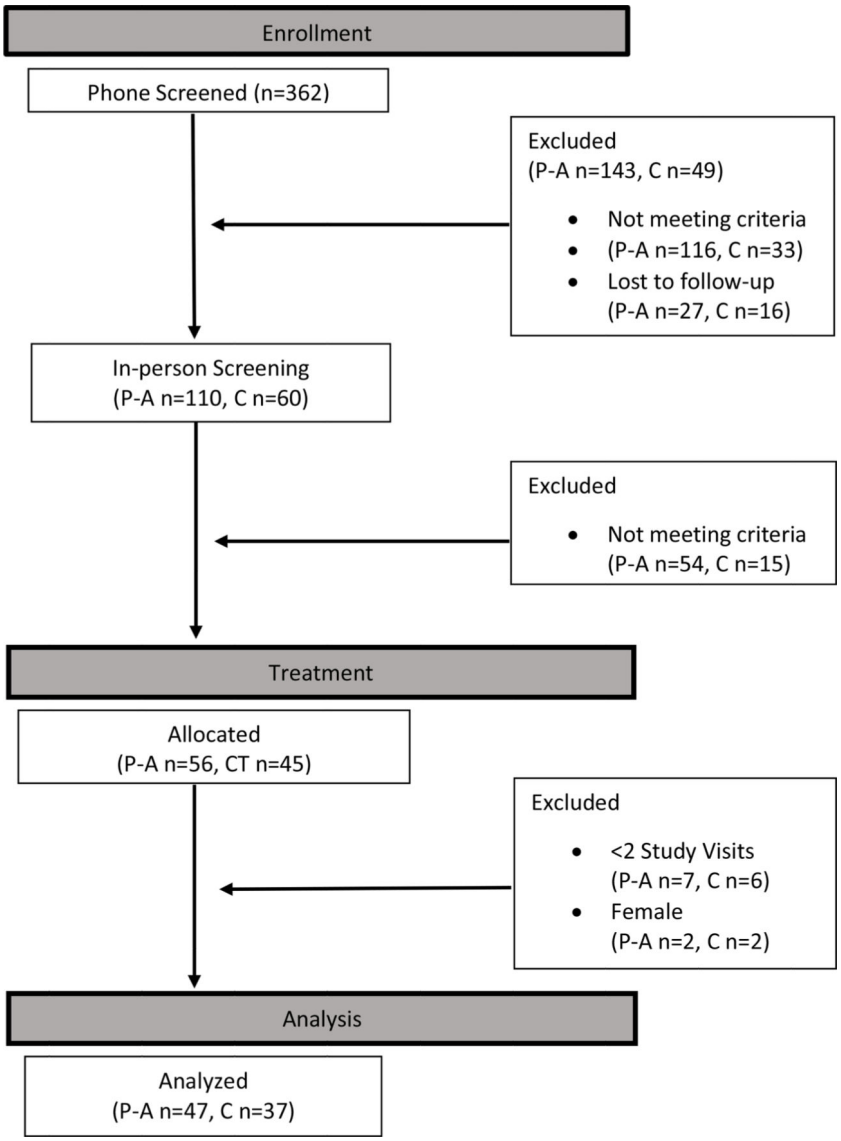

Fig. 1.
Summary flow of participant enrollment. C = control; P-A = PTSD-AUD.

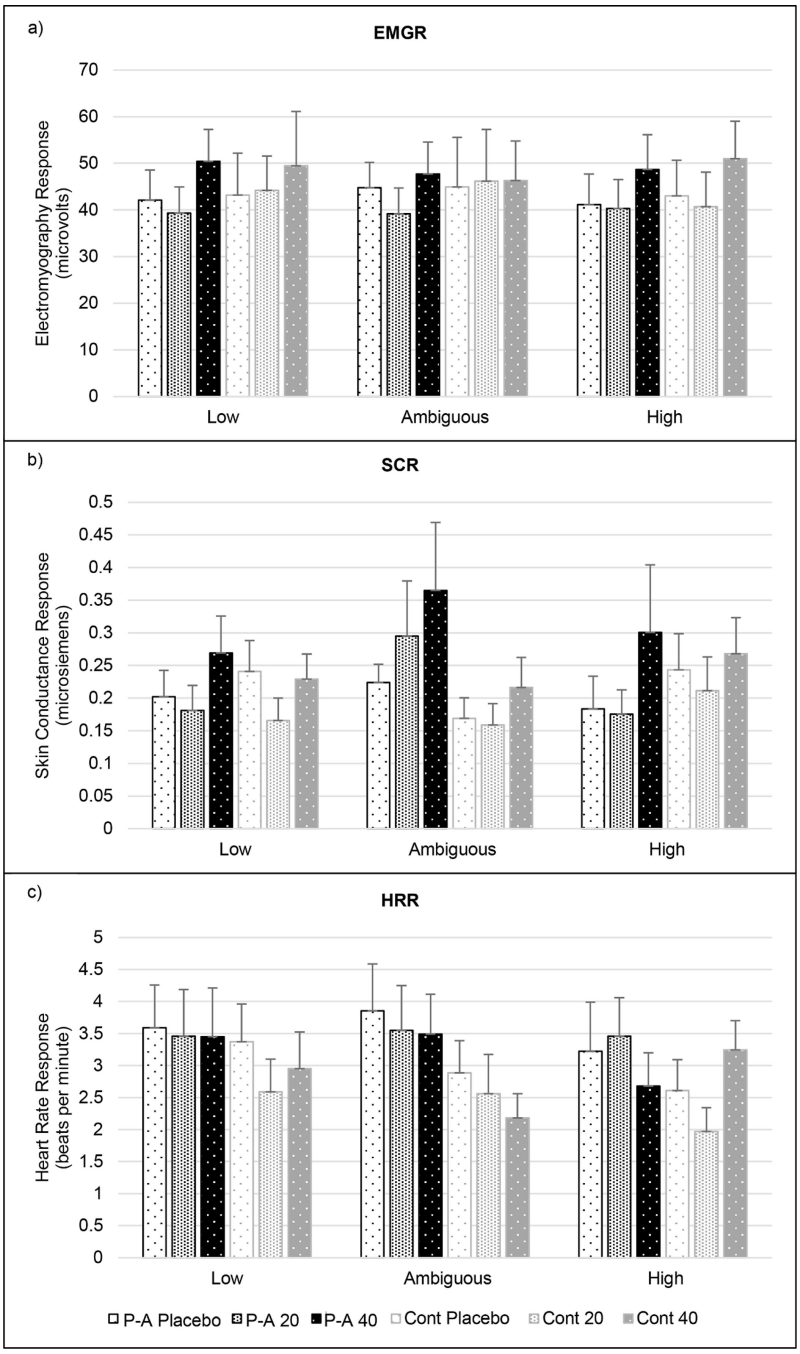

Fig. 2.

Psychophysiology Outcomes. 20 = oxytocin 20 International Units; 40 = oxytocin 40 International Units; Cont = control; EMGR: Electromyography response; HRR = heart rate response; P-*A* = PTSD-AUD; SCR = skin conductance response; y-axes = threat level.

Table 1

Timing of measurements.

	Study Period Screening	Allocation	Experimental Sessions (1, 2, 3)		
			Pre-study drug	*Pre-Startle*	*Post-Startle*
Informed Consent	X				
Eligibility Screen	X				
Drug Order Allocation		X			
Assessments					
Sociodemographic	X				
AUDIT	X				
CAPS-5	X				
SCID-5	X				
Urine Toxicology	X		X		
Acoustic Startle Paradigm					
ECG Recording			X	X	X
EMG Recording			X	X	X
SCR Recording			X	X	X

AUDIT = Alcohol Use Disorders Identification Test, CAPS-5 = Clinician Administered PTSD Scale for *DSM-5*, ECG = electrocardiogram, EMG = electromyography, SCID-5 = Structured Clinical Interview for *DSM-5*, SCR = skin conductance response.

Table 2

Demographics and baseline characteristics.

	PTSD-AUD		Controls	
	(*N* = 47)		(*N* = 37)	
	Mean ± SD		Mean ± SD	
Age, Years	50.6 ± 13.0		48.9 ± 13.9	
Education, Years	14.70 ± 2.51		16.24 ± 2.26	
AUDIT	17.36 ± 8.15		2.25 ± 2.34	
# days used alcohol/past 30 days	0.55 ± 0.37		0.13 ± 0.23	
CAPS-5, Severity Score	34.00 ± 12.31		–	
	n	%	n	%
Gender, Male	47	100	37	100
Race				
Asian	2	4.3	7	18.9
Black	18	38.3	6	16.2
Hispanic	1	2.1	0	0.0
Native American/Pacific Islander	0	0.0	3	8.1
White	17	36.2	17	46.0
Multiracial/Other	9	19.1	4	10.8
In a Relationship	19	40.4	18	48.7
Tobacco Smoker	21	44.7	6	16.2
Veteran	33	70.2	6	16.2

AUDIT = Alcohol Use Disorders Identification Test, CAPS-5 = Clinician Administered PTSD Scale for *DSM-5*, PTSD-AUD = posttraumatic stress disorder and alcohol use disorder group, SD = standard deviation.

Table 3

LMEM outcomes.

			EMGR				SCR				HRR			
	Variable	**Level**	**Effect**	**SE**	**95% CI**	***p***	**Effect**	**SE**	**95% CI**	***p***	**Effect**	**SE**	**95% CI**	***p***
Model 1 (placebo only)	**Group**	(ref. = Control)												
		PTSD-AUD	0.95	9.66	−17.98–19.88	.922	−0.06	0.06	−0.18–0.06	.342	0.62	0.78	−0.92–2.15	.433
	Age		−18.77	9.53	−37.45 – −0.08	.052	**−0.16**	**0.05**	**−0.26–0.07**	**.001**	–	–	–	–
	Relationship		–	–	–	–	**0.14**	**0.04**	**−0.05–0.23**	**.003**	–	–	–	–
	Veteran		–	–	–	–	0.06	0.05	−0.05–0.16	.300	–	–	–	–
Model 2a (overall sample)	**OXT Dose**	(ref. = Placebo)												
		20 IU	−0.98	2.13	−5.16–3.19	.645	−0.01	0.02	−0.06–0.03	.506	−0.29	0.21	−0.69–0.11	.160
		40 IU	**6.33**	**2.14**	**2.13–10.54**	**.003**	**0.06**	**0.02**	**0.01–0.10**	**.010**	−0.27	0.21	−0.67–0.14	.200
	Threat	(ref. = Low)												
		Ambiguous	0.73	2.13	−3.44–4.91	.731	0.03	0.03	−0.01–0.08	.152	−0.11	0.21	−0.51–0.29	.596
		High	−0.70	2.15	−4.92–3.53	.747	0.01	0.02	−0.03–0.06	.605	−0.39	0.21	−0.79–0.02	.063
	Age		**−19.54**	**8.82**	**−36.82 – −2.25**	**.030**	**−0.23**	**0.06**	**−0.35 – −0.11**	**<0.001**	–	–	–	–
	Relationship		–	–	–	–	**0.18**	**0.06**	**0.06–0.29**	**.003**	–	–	–	–
	Veteran		–	–	–	–	0.12	0.06	−0.002–0.23	.057	–	–	–	–
Model 2b (PTSD-AUD only)	**OXT Dose**	(ref. = Placebo)												
		20 IU	−2.81	2.60	−7.91–2.29	.282	0.01	0.04	−0.06–0.08	.746	−0.07	0.28	−0.62–0.48	.801
		40 IU	**6.05**	**2.62**	**0.90–11.19**	**.022**	**0.10**	**0.04**	**0.03–0.17**	**.007**	−0.35	0.28	−0.90–0.20	.213
	Threat	(ref. = Low)												
		Ambiguous	1.87	2.60	−3.23–6.97	.473	**0.09**	**0.04**	**0.02–0.17**	**.012**	0.13	0.28	−0.41–0.68	.636
		High	−0.08	2.65	−5.27–5.11	.977	0.02	0.04	−0.06–0.09	.643	−0.38	0.28	−0.93–0.17	.171
	Age		−20.82	10.91	−42.20–0.56	.062	**−0.33**	**0.10**	**−0.53 – −0.13**	**.002**	–	–	–	–
	Relationship		–	–	–	–	**0.20**	**0.09**	**0.01–0.38**	**.043**	–	–	–	–
	Veteran		–	–	–	–	0.12	0.10	−0.08–0.33	.256	–	–	–	–
Model 2c (control only)	**OXT Dose**	(ref. = Placebo)												
		20 IU	1.38	3.53	−5.54–8.30	.696	**−0.05**	**0.02**	**−0.09 – −0.0007**	**.048**	−0.57	0.30	−1.17–0.02	.060
		40 IU	6.80	3.55	−0.15–13.75	.056	0.01	0.02	−0.04–0.06	.675	−0.16	0.30	−0.75–0.44	.601
	Threat	(ref. = Low)												

			EMGR				SCR				HRR			
	Variable	**Level**	**Effect**	**SE**	**95% CI**	***p***	**Effect**	**SE**	**95% CI**	***p***	**Effect**	**SE**	**95% CI**	***p***
		Ambiguous	−0.77	3.53	−7.68–6.15	.828	−0.03	0.02	−0.08–0.01	.151	−0.42	0.30	−1.01–0.17	.165
		High	−1.52	3.54	−8.46–5.42	.668	0.01	0.02	−0.04–0.05	.713	−0.39	0.30	−1.09–0.21	.201
	Age		−18.00	14.38	−46.19–10.19	.219	**−0.12**	**0.06**	**−0.23 – −0.01**	**.042**	–	–	–	–
	Relationship		–	–	–	–	**0.16**	**0.05**	**0.05–0.26**	**.007**	–	–	–	–
	Veteran		–	–	–	–	0.08	0.08	−0.08–0.23	.328	–	–	–	–

Effect = per unit effect size (regression coefficient); CI = confidence interval; EMGR = electromyography response; HRR = heart rate response; IU = international units; OXT = oxytocin; PTSD-AUD = posttraumatic stress disorder and alcohol use disorder; SCR = skin conductance response; SE = standard error; Bold = p-value < 0.05.