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# IMPACT OF PROPHYLACTIC INTRANASAL OXYTOCIN ADMINISTRATION ON SYMPTOMS OF POST-TRAUMATIC STRESS

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**Abstract**

Post-traumatic stress disorder (PTSD) is a mental health condition that affects people after instances of severe emotional trauma. Research suggests that oxytocin treatment decreases PTSD symptoms. This study served to evaluate the efficacy of intranasal oxytocin pre-treatment on symptoms related to PTSD. The hypotheses are that oxytocin will decrease fear and anxiety, and increase reward-seeking behaviors. Sprague Dawley rats were assigned to three groups (*Control, Stress*, *Oxytocin*, and *Oxytocin+Stress*; n=6 per group) to conduct this experiment. Prior to foot shock treatment, rats were trained to expect a food reward (Kellogg's Froot Loops) in an open field enclosure. Subsequently, the *Oxytocin* and the *Oxytocin+Stress* groups were pre-treated with intranasal oxytocin and then the *Stress* and *Oxytocin+Stress* groups were exposed to an inescapable foot shock (a model PTSD inducing stressor). After oxytocin and shock treatments, rats underwent various behavioral tests: re-exposure to the shock chamber to assess fear, elevated O-maze to assess anxiety, and food reward trials in the open field enclosure to assess reward-seeking behavior. The oxytocin treatment decreased fear related symptoms upon re-exposure to the fear conditioning chamber; both colonic motility and freezing time were lower in the *Oxytocin+Stress* group compared to the *Stress* group. The foot shock model failed to produce significant behavioral changes related to anxiety and reward-seeking behavior between the *Control* and *Stress* groups.

## Background and Significance

### PTSD

Post-traumatic stress disorder (PTSD) is a mental health condition that affects people after instances of severe emotional or physical trauma. Diagnostic criteria are complex and symptoms vary between individuals, but include emotional distress, physical reactivity, avoidance of trauma related reminders, decreased interest in activities, and difficulty experiencing positive affect (American Psychiatric Association, 2013). It is estimated that between 8.3% and 9.4% percent of the United States population develops diagnosable PTSD in their lifetime (Kilpatrick et al., 2013).

### Rodent Model of PTSD

While previous researchers have used corticosterone administration to induce symptoms of PTSD in rodents (Levy et al., 2001), more recently a fear-conditioning paradigm using chronic electric foot shock (described in the Methods section) has been developed to induce PTSD-like symptoms in a rat model (Sahraei et al., 2012; Yu et al., 2012; Gao et al., 2014) . This paradigm produces behavioral changes in rats such as fear and anxiety, which are known to be associated with PTSD.

Fear in rats is commonly assessed by increased freezing behavior (Fanselow, 1994; Sahraei et al., 2012; Yu et al., 2012; Gao et al., 2014). Freezing is defined as motionless periods upon re-exposure to the previously traumatizing source of stimuli, the foot shock chamber (Yu et al., 2012; Gao et al., 2014), and is a hallmark of stress-induced behavioral changes in rodents. Another assessment of fear in rodents is increased defecation when re-exposed to fear related stimuli (Lester et al.,

1968; Gao et al., 2011). Increased defecation, often referred to as "colonic motility", is a measure highly sensitive to psychological stress (Stam et al., 1995). Colonic motility is thought to be regulated by the release of corticotropin-releasing hormone caused by stressful events (Verleye & Gillardin, 2004).

Anxiety is commonly manifested and measured in rodents as decreased locomotion (Fernandes et al., 1999; Prut & Belzung, 2002) and increased time in enclosed spaces (Pellow et al., 1985; Shepherd et al., 1994; Ennaceur et al., 2006). Many different methods have been used to assess anxiety in rodents and include the open-field test, elevated plus-maze, and elevated O-maze (reviewed in Sestakova et al., 2013). The open-field test is the simplest and longest used anxiety test for rats, dating back to 1930's (Hall and Ballachey, 1932). While specific requirements of the open-field are not standardized, in general it consists of an open area in which ambulation can be observed (Walsh and Cummings, 1976). The test measures locomotion and propensity to explore; lower amounts of locomotion and exploration correlate to higher levels of anxiety (Perals et al., 2017; Prut and Belzung 2003). The elevated plus-maze is a four-armed maze that is raised off the ground by legs. Each arm is connected at a center platform. Two arms of the plus-maze have high walls and the other two arms do not have walls. The time spent in the walled areas is associated with anxious behavior (Pellow et al., 1984; Rodgers and Dalvi, 1997) as the rat is thought to be avoiding the novelty of the open area (Dawson & Tricklebank, 1995). The elevated O-maze (or elevated zero-maze) was developed as an improvement to the elevated plus-maze. The concepts of the plus-maze, such as height off the ground and the open versus closed areas, remain the

same but the O-maze forms a continuous circle (or zero) with alternating quadrants of open (no walls) and closed (walled off) areas. This configuration allows for easier exploration between maze sections since there are no corners and the rats do not have to turn around while continuing to move about the maze (Shepherd et al., 1994). It has been suggested that the elevated O-maze produces more consistent results over time (Tucker & McCabe, 2017); however, the results of the elevated O-maze are diminished by daily re-exposure (Cook et al., 2001).

Anhedonia (the decrease in the capacity to feel pleasure) has relatively recently been incorporated into the diagnostic criteria of PTSD (Stein et al., 2014). It is thought that PTSD may be associated with reward-seeking impairments. While the response is varied, there seems to be an overall decrease in reward anticipation, approach (or wanting), and hedonic responses to reward (Nawijn et al., 2015). This symptom of PTSD, observed in humans, has not yet been investigated in the rodent model. Reward seeking in rodents is most commonly assessed by the use of an operant box (Dingess et al., 2017; Piantadosi et al., 2017). Since operant boxes are currently not available in our lab, one of the goals of this study was to investigate the impact of stress on the reward-seeking behavior in an open field circular enclosure (described in the Methods section). Furthermore, a recent study conducted by Nawijn et al. (2016) suggests that oxytocin treatment in humans with PTSD appears to impact the brain regions involved in reward processing. Therefore, another goal of this study was to investigate the impact of oxytocin on PTSD-related behaviors (i.e., fear, anxiety, and reward-seeking).

**Oxytocin**

Oxytocin is a neurohormone produced by neurons of the paraventricular and supraoptic nuclei in the hypothalamus. It is associated with many functions of the body including human emotion and motivation (Love, 2014). Administered after traumatization, oxytocin has been shown to reduce symptoms associated with PTSD (i.e., fear and anxiety) in both rodent (Missig et al., 2010; Ayers et al., 2011; Zoicas et al., 2014; Janezic et al., 2016; Sack et al., 2017) and human clinical trials (Bakermans-Kranenburg & Van IJzendoorn, 2009; Acheson et al., 2013; Frijiling et al., 2014).

Oxytocin is a neuropeptide that directly interacts with oxytocin receptors in specific parts of the central nervous system, as such it is considered a neuromodulator in brain regions associated with fear, aggression and social behaviors (Febo & Ferris, 2014; Heinrichs & Domes, 2008). Oxytocin receptors are expressed in the amygdala, which is an area of the brain intimately involved in processing of emotion and cognition (reviewed in Phelps, 2006). Furthermore, oxytocin receptors are also found in reward processing areas of the brain (Febo and Ferris, 2014) including the ventral tegmental area and the nucleus accumbens (Wise and Bozarth, 1987; Nicola, 2016). Hypothalamic oxytocin neurons have direct axonal connections to the amygdala, ventral tegmental area, and nucleus accumbens and are thought to directly modulate the activity of these brain regions (Bethlehem et al., 2012).

In addition to its direct effects on brain regions associated with the processing of emotions and motivation, oxytocin also interacts with the hypothalamic-pituitary-adrenal (HPA) axis. This endocrine feedback system is

implicated in stress reactions as well as regulating many body processes (Bhatnagar et al., 2006; Stranahan et al., 2008; Hall et al., 2012; Daskalakis et al., 2013). The HPA axis can be modified permanently by early childhood trauma, rendering it hyper reactive (van Bodegom et al., 2017). Oxytocin has been shown to inhibit stress responses associated with the HPA axis such as corticosterone release (Windle et al., 1997; Heinrichs et al., 2003; De Kloet et al., 2006). As a natural mechanism, oxytocin offers protection to stress associated with the HPA axis in postpartum, breastfeeding mothers (Cox et al., 2014). Oxytocin levels rise peripherally following stressful incidents and higher oxytocin levels correspond to faster recovery from stress related symptoms (Engert et al., 2016). Therefore, oxytocin treatment could potentially provide neuroprotection during stressful events.

**Prophylaxis**

Various pharmaceutical and psychosocial interventions have been studied as potential preventative treatments for PTSD (reviewed in Baker et al., 2009; Daskalakis et al., 2013). Most preventative measures that have been explored fall into the category of "early intervention" in which treatment is given after the traumatic event, but prior to development of PTSD related symptoms (reviewed in Birur et al., 2017). Multiple drugs have been studied as potential preventative treatments for PTSD (Vaiva et al., 2003; Baker et al., 2009; Daskalakis et al., 2013, Morena et al., 2017). However, the efficacy of oxytocin as a prophylactic, or preventative, treatment has been minimally explored in humans (Frijling et al., 2014; van Zuiden et al., 2017) and there is a dearth of research with rodents

(Renicker et al., 2015). Oxytocin may offer the same neuroprotective benefits of other treatments (Vaiva et al., 2003; Baker et al., 2009; Daskalakis et al., 2013, Morena et al., 2017) without the risks associated with long-term treatment, including antidiuresis and hyponatremia (Baker et al., 2009).

**Intranasal Administration**

Intranasal administration of oxytocin is an effective therapeutic delivery method in humans (Fischer-Shofty et al., 2010; Guastella et al., 2010; Acheson et al., 2013; Nawijn et al., 2016). While the mechanisms are not clearly understood, intranasal oxytocin administration produces "clear and specific changes in neural activation" (Veening & Olivier 2013) and has been shown to increase levels of oxytocin in cerebral spinal fluid (Stevens et al. 2013, Streipens et al., 2013). Intranasal oxytocin administration has also been shown to impact specific brain regions considered 'social' regions (Bethlehem et al., 2012). Oxytocin is a very small peptide of nine amino acids (a nonapeptide) and is believed to at least partially pass through the blood brain barrier (Ermisch et al., 1985). Oxytocin administrated intranasally is thought bypass the blood brain barrier (Talegaonkar & Mishra 2004) and has been shown to produce higher levels of oxytocin in the brain than peripheral administration (Neumann et al., 2013). Additionally, peripheral oxytocin may have different effects on stress than oxytocin delivered directly into the central nervous system via intranasal administration. One study found that increased

plasma oxytocin levels correlate to increased cortisol levels (Taylor et al., 2006), although causation was not implied. Research suggesting that intranasal administration of oxytocin directly and effectively impacts brain function warrants investigation into its potential therapeutic effects on conditions such as PTSD.

The purpose of this study was to test the hypothesis that prophylactic oxytocin treatment with decrease PTSD related symptoms in a rat model, specifically decreasing fear and anxiety related behaviors and increasing reward-seeking behaviors.

## Methods

Twenty-four male Sprague Dawley rats were randomly assigned to four groups (n=6, per group): 1. *Control* group (no shock and no oxytocin treatment), 2. *Stress* group (exposed to shock and no oxytocin treatment), 3. *Oxytocin* group (no shock and treated with oxytocin), and 4. *Oxytocin+Stress* group (treated with oxytocin and exposed to shock). For practical purposes the rats were split into 3 cohorts, testing n=2 rats from each treatment group at a time (2-3 month timeframe).

### General housing

Rats were housed individually to most effectively monitor food consumption during behavioral tasks. Rats were housed in a polysulfone filter top cage. Cages contained corn cob bedding**,** PVC pipe, and water supplied *ad libitum*. "Harlan 2018" food was provided *ad libitum*, except during the weeks of food deprivation

(described below). All rats were housed in the same room kept at a temperature of 22 ± 1°C, and a humidity of 23-33% with a 12 hour light/dark cycle.

**Reward Training**

Prior to oxytocin treatment and fear conditioning (foot shock), all rats were pre-trained to expect and retrieve a food reward (Kellogg's Froot Loop) in a circular open field enclosure (3-ft x 3-ft, 1-ft walls) with a reward delivery tube at one end (Fig. 1). For one week, rats were habituated to the enclosure by allowing them to roam freely for a minimum of 5-10 minutes a day (Monday-Friday), three Froot Loops were delivered into the chamber during this time to familiarize them to the reward delivery procedure and assess their interest in the food reward. For the next 2-3 weeks rats were food restricted, placed in a start box in the open field enclosure and 3 Froot Loops were delivered independently into the reward-seeking area (Fig. 1). For each reward-seeking trial, the rat was placed in the start box, the Froot Loop was delivered and the start box door was opened manually by the experimenter. The time for the rat to retrieve the reward (within a minute of reward delivery) was monitored and recorded as an assessment of their "reward-seeking" behavior.

**Food Deprivation**

After the first week of habituation, rats were food restricted on Monday-Friday and fed *ad libitum* on the weekends. Rats were weighed daily while food restricted and given an amount of food that correlated to their weight change. Rats who lost 0-5 grams, or gained weight were fed one half of a food pellet, those who lost 6-10 grams were fed 1 pellet, rats who lost 11-15 grams were fed 1.5 pellets and

those who lost 15 grams or more were fed 2 pellets. Rats who drop below 80% of their starting weight during food restriction would have been fed *ad libitum,* though there were no instances of this in our study.

**Oxytocin Administration**

Rats were lightly anesthetized with isoflurane to calm them sufficiently to allow for intranasal administration. The *Oxytocin+Stress* and the *Oxytocin* groups were treated with intranasal oxytocin at 0.1 µL/kg, 30 minutes prior to fear conditioning (described below) based on Ayers et al. (2011) procedure. The *Control* and *Stress* groups were administered an equivalent amount of saline. The rats were then assessed for fear, anxiety and reward-seeking behaviors (described below).

**Fear Conditioning**

The *Stress* and *Oxytocin+Stress* groups were exposed to an electric foot-shock paradigm (used as a model for a PTSD inducing stressor; Gao et al, 2014). Over a period of three days, rats in these groups were exposed to foot shock twice daily. They were placed into the fear-conditioning chamber twice daily and given 20 inescapable foot shocks (8 mA intensity, 3 second duration, 10 second intervals between shocks; similar to Gao et al., 2014).

**Behavioral Assessments**

After oxytocin and shock treatments, all rats were reintroduced to the shock chamber to assess behaviors related to fear (i.e., time motionless and defecation), run on an elevated O-maze (Fig. 2) to assess behaviors related to increased anxiety

(i.e., decrease in time spent in the open segments of the maze), and given three reward delivery trials in the open field enclosure to assess reward-seeking behavior (i.e., time to retrieve reward).

Rats were placed in the shocking chamber for five minutes, but not shocked, to observe fear related behaviors. Fear is often expressed and measured by an increase in freezing time (defined as the absence of all movements except for those related to respiration; Gao et al. 2014) and by an increase in fecal production (Gao et al., 2011) when reintroduced into the fear conditioning (shocking) chamber. Therefore, the rats were put back into the shocking chamber for 5 minutes and were observed for freezing time and the amount of fecal production.

To assess anxiety, the rats were placed in an elevated O-maze for five minutes, measuring the amount of time spent in the open segments which is inversely correlated to anxiety level of the animal as proposed by Shepherd et al. (1994). The elevated O-maze (Fig. 2) consisted of an annular platform elevated 65 cm above the floor (105 cm in diameter, 10 cm in width), the platform of the elevated O-maze was divided into 4 segments: 2 opposing open segments with no walls, and 2 opposing closed segments with walls extending 27 cm above the platform surface. The O-maze was located in an otherwise empty room and the researcher stepped out of room and closed the door once rat was placed in the open segment of the O-maze as to not distract from the rats normal exploratory behavior. Behavior was recorded on video for later analysis.

To assess reward-seeking behavior, the rats were then placed in the open field enclosure (Fig. 1) for 3 consecutive 1-minute trials, based on the reward training procedure. At the beginning of each trial, the rat was enclosed in a start box. The start box door was opened and a Froot Loop (food reward) delivered through the reward delivery tube. The time to retrieve the Froot Loop was measured. Behavior was recorded on video for later analysis.

All behavioral tests were done twice: week 1 (anxiety tested 1 day after shock treatment, fear and reward-seeking tested 2 days after shock treatment) and again in week 2 (anxiety tested 8 days after shock treatment, fear and reward-seeking tested 9 days after shock treatment).

**Statistical analysis**

To determine significant differences in behavioral (dependent) measures, we first compared data from all four groups using a *one-way ANOVA (VassarStats)*. If overall significance ($p \leq 0.05$) was found, a subsequent *Tukey's post-hoc* analysis was used to determine significant differences between individual groups. Standard two-tailed t-tests *(VassarStats)* were used to analyze changes within groups between week one and two. We were particularly interested in the differences between the *Stress* and *Stress+Oxytocin* groups to most directly address the objectives of this study.

*The procedures described in this proposal have been reviewed and approved by the Eastern Washington University Institutional Animal Care and Use Committee (effective June 7, 2016).*

## Results

For behavioral measures assessing fear, we found overall significance in both measures (freezing and defecation), during both week 1 and week 2 (Fig. 3, $p<0.0001$; Fig. 4, $p<0.001$; Fig. 7, $p<0.0005$; Fig. 8, $p<0.05$). Freezing time in seconds was significantly lower for *Control*, *Oxytocin*, and *Oxytocin+Stress* groups when compared to the *Stress* group (Fig. 3, $p<0.01$) during week 1. In week 2, the decreased freezing time between the *Stress* group and the *Oxytocin* and *Control* groups remained significant (Fig 7, $p<0.01$) but the *Oxytocin+Stress* group was no longer significantly lower than the *Stress* group, or significantly higher than the *Control* group (Fig 7). When comparing week 1 to week 2, the freezing times for the *Stress* group shows a significant decrease over time (Fig.11, $p<0.05$) while this decrease for the *Oxytocin+Stress* group was not significant across the two weeks (Fig. 11).

Fecal Production (measured in grams of feces) was significantly lower for *Control*, *Oxytocin*, and *Oxytocin+Stress* groups when compared to the *Stress* group (Fig. 4, $p<0.05$) in week 1 after fear conditioning. By week 2 the *Stress* group was still significantly higher than the *Oxytocin* and *Control* groups (Fig 8, $p<0.01$) but the *Oxytocin+Stress* group was no longer significantly lower than the *Stress* group, or significantly higher than the *Control* group (Fig 8).

Tests for anxiety and reward seeking behavior failed to produce significant results in either week 1 or week 2 (overall *one-way ANOVA*; Fig. 5, $p=0.15$; Fig. 6, $p=0.47$; Fig. 9, $p=0.68$; Fig. 10, $p=0.55$).

## Discussion

It is clear that the PTSD paradigm worked to induce fear in the rats based on the differences between the *Control* and *Stress* groups (Fig. 3-4, Fig.7-8). These results also supported our hypothesis that prophylactic oxytocin treatment would decrease fear related behaviors after a PTSD-like stressor. While the *Oxytocin+Stress* group had increased fear related behaviors compared to the *Control* group, both the time spent freezing (Fig. 3) and the amount of fecal production (Fig. 4) were decreased significantly compared to the *Stress* group. However, the significant difference in fear related behaviors between the *Stress* and *Oxytocin+Stress* groups does not continue in the second week post stressor (Fig. 7-8). This lack of significance could be due to the attenuation of fear responses over time in the *Stress* group which decreased from week one to week two, as opposed to diminished efficacy of the prophylactic treatment in the *Oxytocin+Stress* group, which also decreased in fear related measures but were not significant (Fig. 11).

As there was no significance in anxiety related behaviors between the *Control* and the *Stress* groups (Fig. 5 and Fig. 9), it appears our paradigm did not work for testing or invoking these symptoms. It is unclear why the elevated O-maze did not produce results as it has shown to be reliable in other similar tests of the PTSD-like model (Gao et al., 2014; Renicker et al., 2015). Gao et al. (2014) who developed the PTSD-like model in rats used an elevated plus-maze to test anxiety so their results cannot be compared directly to our O-maze results, although a similar effect would be expected. One possible explanation is the temporal sequence of testing, Gao et al.

(2014) performed the elevated plus-maze test after re-exposure to the fear conditioning chamber while our elevated O-maze tests were done the day before re-exposure. Renicker et al. (2015) also tested the elevated O-maze after re-exposure to the fear conditioning chamber, although there was a gap of time (5 days) between the tests. Previous research has suggested that a reminder of the stress experience can influence memory and behavior (Zoladz et al., 2010; Burke et al., 2013). As such, it is possible that the re-exposure to the fear-conditioning chamber enhanced the anxiety response seen in the previous studies of Gao et al. (2014) and Renicker et al. (2015).

There was also a lack of significance in reward-seeking behaviors between the *Control* and the *Stress* groups (Fig. 6 and Fig. 10). Since reward-seeking behaviors have not been analyzed in the rodent model of PTSD, it is possible that these behaviors are not affected in rodents as is found in human cases of PTSD. Alternatively, since the reward-seeking paradigm used in this study was developed in our lab as a cost effective substitute to the classical operant box, it is possible that these reward-seeking behaviors are impacted in the rat model of PTSD but our dependent measures did not expose these effects. If this experiment were to be repeated, it might benefit from the use of operant boxes as has been used in previous studies to assess reward-seeking behaviors (Dingess et al., 2017; Piantadosi et al., 2017).

An important aspect to consider is the timing of "prophylaxis". While our study defined prophylaxis as treatment prior to the traumatic stressor, other studies

define preventative treatment as interventions performed soon after the traumatic event before symptoms of PTSD manifest, also called "early intervention" (Vaiva et al., 2003; Baker et al., 2009; Daskalakis et al., 2013; Rothbaum et al., 2014; Frijling et al., 2014; Renicker et al., 2015). Early intervention or prophylactic treatments could also be affecting different mechanisms than traditional treatments, such as memory consolidation (Morena et al., 2017). Future studies should evaluate the pros and cons of each method. Treatment after the traumatic event would be most practical as the vast majority of traumatic events cannot be predicted; however, if treatment before a traumatic stressor offers significant protection it could be beneficial in settings such as combat warfare, law enforcement operations, and surgical settings.

Whether oxytocin treatment is delivered soon after the traumatic event (Frijling et al., 2014; Renicker et al., 2015; van Zuiden et al., 2017) or before, as was done in this study, research suggests that intranasal oxytocin administration provides neuroprotection and attenuates fear related behaviors in humans (Frijling et al., 2014; van Zuiden et al., 2017) and rodents (Renicker et al., 2015). The direct effects of oxytocin on brain regions that contain oxytocin receptors and process emotion (e.g., amygdala) are likely implicated in the behavioral changes we observed in the measures of fear. Furthermore, PTSD has been shown to alter endocrine output related to the HPA axis (reviewed in Daskalakis et al., 2013). Intranasal oxytocin administration is likely impacting the HPA axis but how oxytocin alters the HPA axis is poorly understood (reviewed in Stockhorst and Antov, 2016).

Certain factors should be considered when designing future studies. Genetics and epigenetics both play a role in the likelihood of developing PTSD (Yehuda & Bierer 2009; Gerritsen et al., 2017) as well as the potential for treatment success (Yehuda et al., 2013). Additionally, sex differences are found to be very pronounced in the instances of PTSD (2:1 female to male ratio) (Kilpatrick et al., 2013) and oxytocin efficacy (Sack et al., 2017; Smith & Wang 2013). As our study population consisted of only male rats we were not be able to address these differences. Many factors influence the probability of developing PTSD. In humans, while approximately 80% of the population is exposed to traumatic stressors, only a small percentage (~11%) of them go on to develop the disorder (Kilpatrick et al., 2013). Therefore, a relatively small study such as ours may not see a large enough sample of affected individuals to gain an accurate representation of the effects.

The results of this study suggest that further testing is warranted to ascertain whether or not prophylactic oxytocin treatment is an effective and practical prevention method for PTSD. Our fear results corroborate the findings of van Zuiden et al. (2017) Friljing et al. (2014) and Renicker et al. (2015); however, the impact of prophylactic oxytocin treatment on anxiety and reward seeking need further investigation.

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## Figures

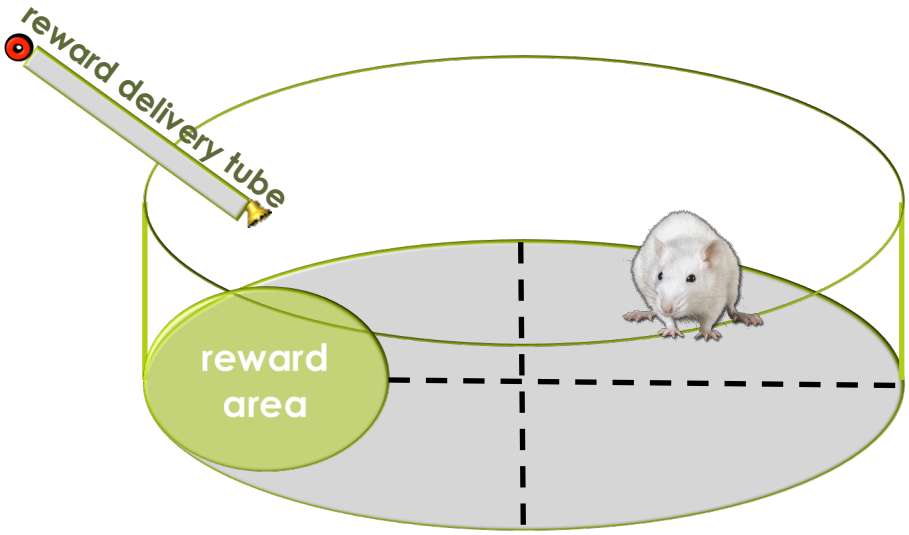


**Figure 1.** Schematic diagram of open field enclosure and reward delivery area.


**Figure 2.** Rat in the enclosed area of the elevated zero maze.

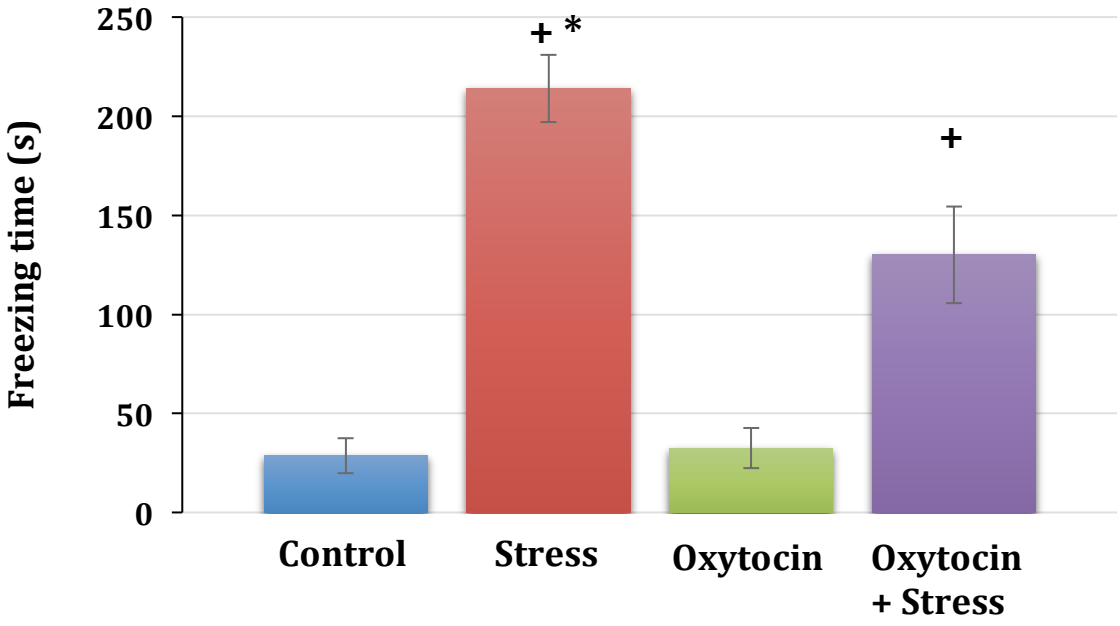


**Figure 3.** Time spent motionless (freezing) during 5 minute re-exposure to the foot shock chamber following oxytocin *pretreatment* (n=6, per group) week one post stress. Overall significant difference by ANOVA $p<0.0001$
* significantly different than Oxytocin+Stress $p<0.05$;
+ significantly different than Control and Oxytocin $p<0.05$

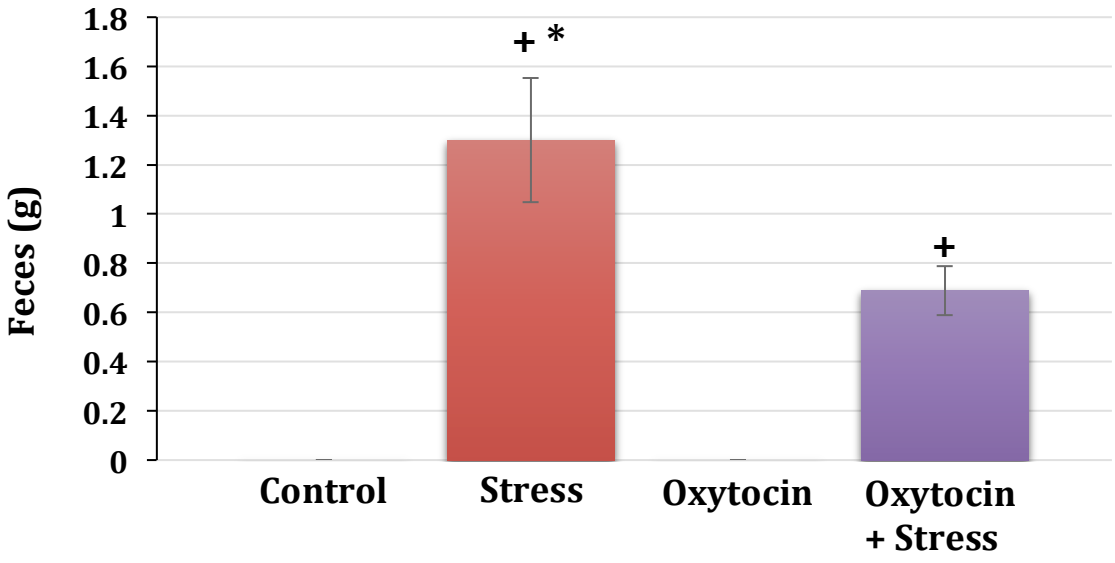


**Figure 4.** Fecal production during 5 minute re-exposure to the foot shock chamber following oxytocin *pretreatment* (n=6, per group) week one post stress. Overall significance by ANOVA $p<0.001$;
* significantly different than Oxytocin+Stress $p<0.05$;
+ significantly different than Control and Oxytocin $p<0.05$

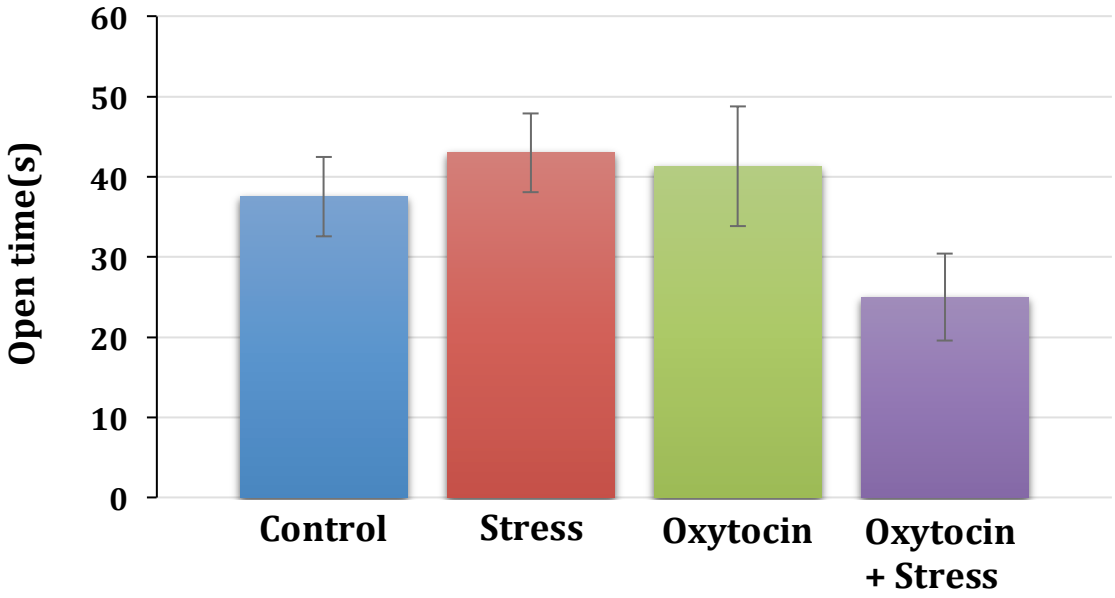


**Figure 5.** Time spent in the open arm during 5-minute exposure to the elevated zero maze following oxytocin *pretreatment* (n=6, per group) week one post stress. There was no significant difference between the groups $p=0.15$

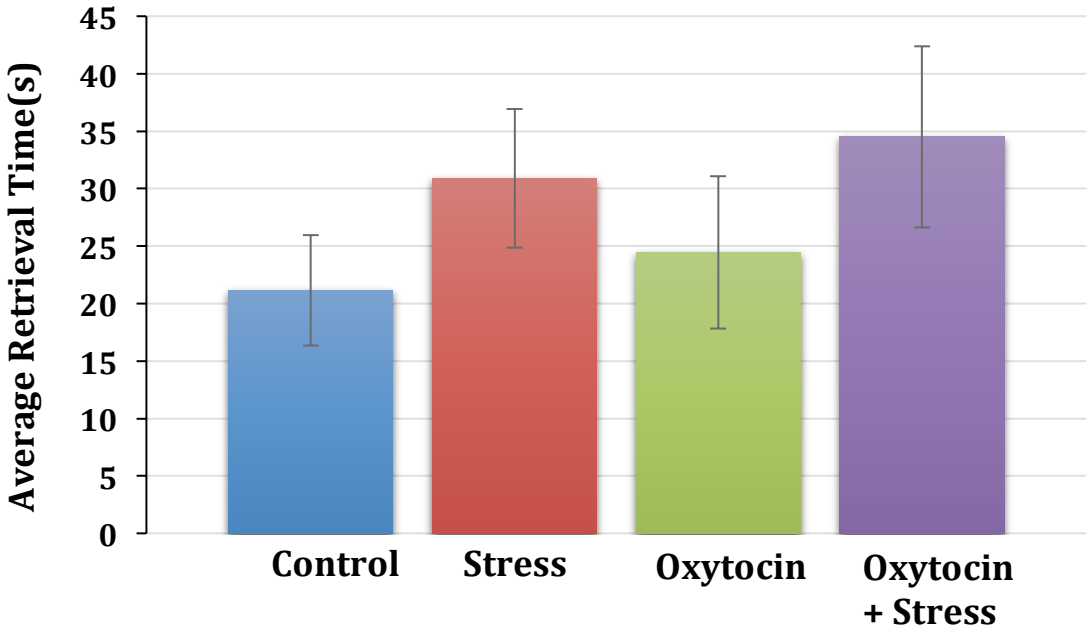


**Figure 6.** Average time across 3 trials to retrieve food reward (Froot Loop) in open field enclosure following oxytocin *pretreatment* (n=6, per group) week one post stress. There was no significant difference between groups p=0.47

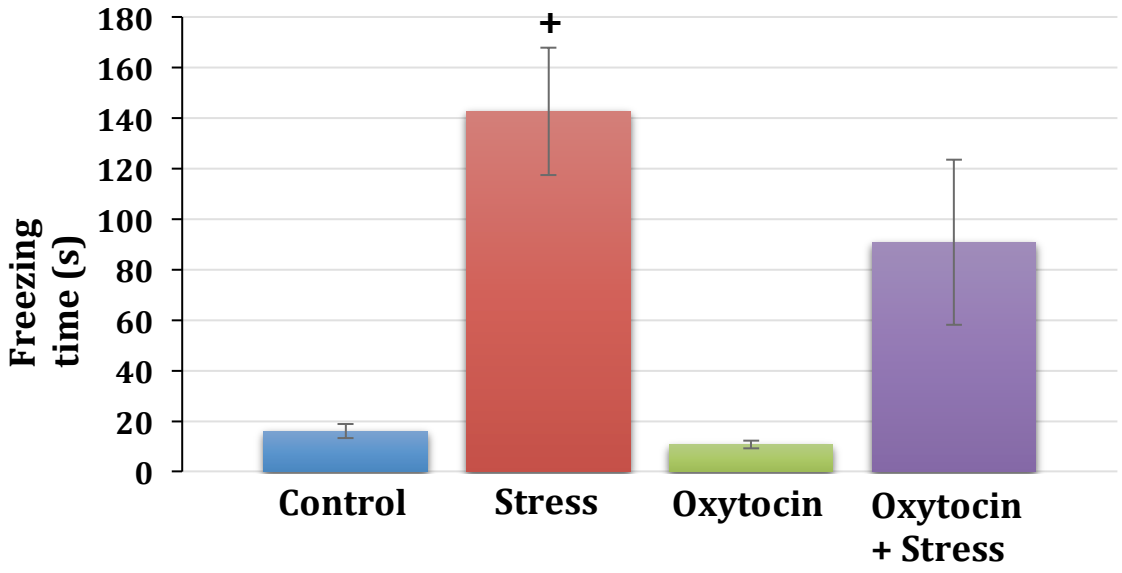


**Figure 7.** Time spent motionless (freezing) during 5-minute re-exposure to the foot shock chamber following oxytocin *pretreatment* (n=6, per group), week two post stress. Overall significance from ANOVA p<0.0005

+ significantly different than Control and Oxytocin p<0.05

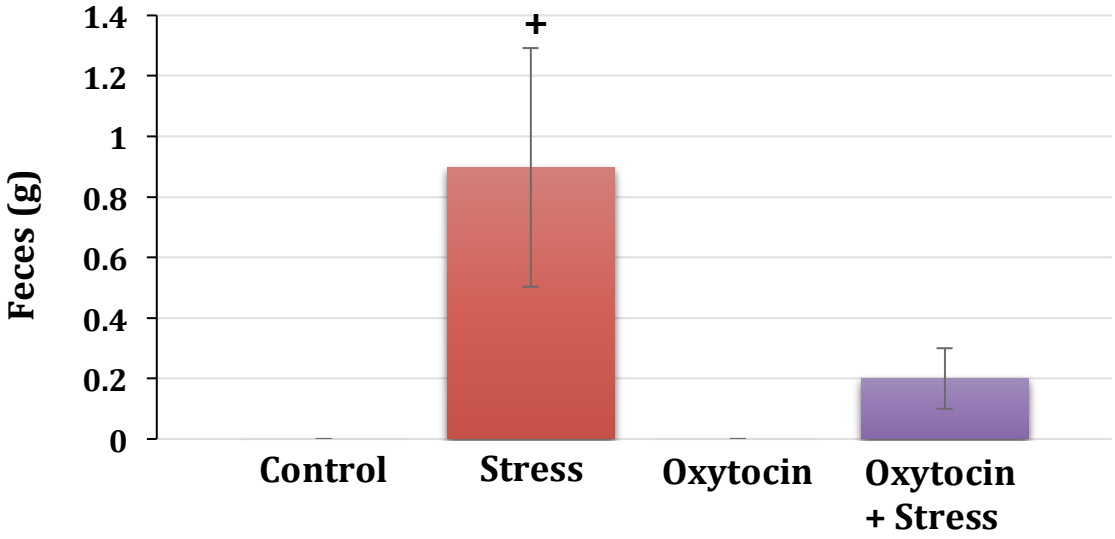


**Figure 8.** Fecal production during 5-minute re-exposure to the foot shock chamber following oxytocin *pretreatment* (n=6, per group), week two post stress. Overall, p=0.016;
+ significantly different than Control and Oxytocin p<0.05

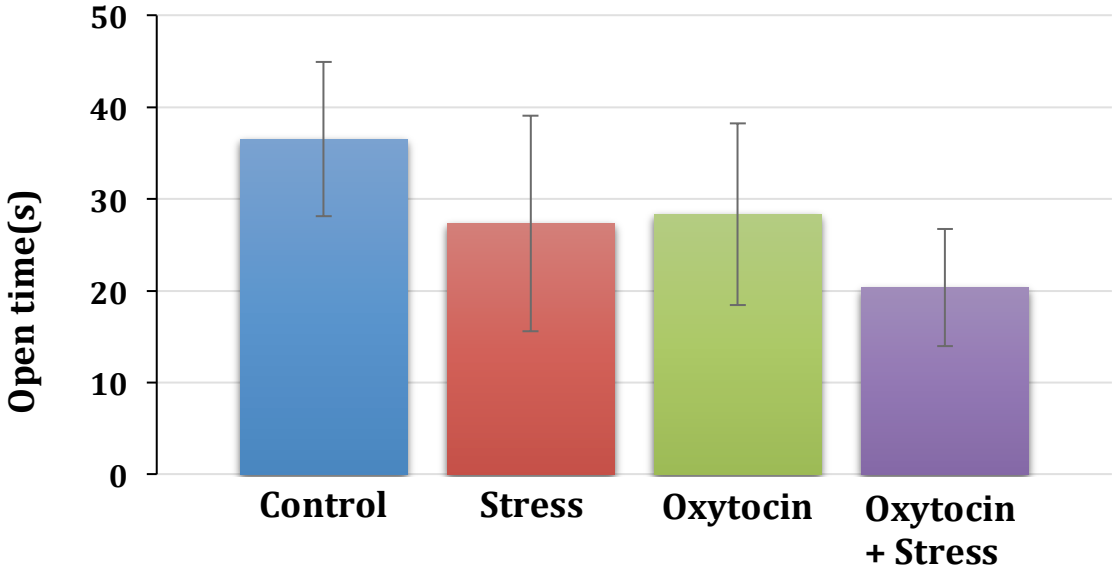


**Figure 9.** Time spent in the open arm during 5 minute exposure to the elevated zero maze following oxytocin *pretreatment* (n=6, per group) two weeks post stress. There was no overall significance between groups p=0.68.

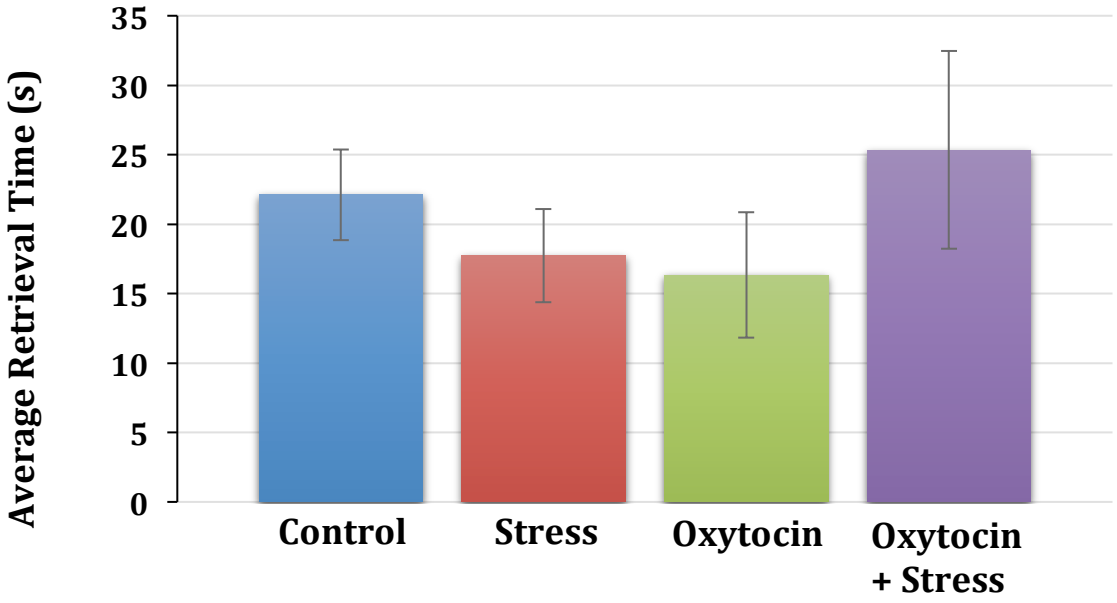


**Figure 10.** Time to retrieve food reward (Froot Loop) in open field enclosure following oxytocin *posttreatment pretreatment* (n=6, per group) two weeks post stress. p=0.55

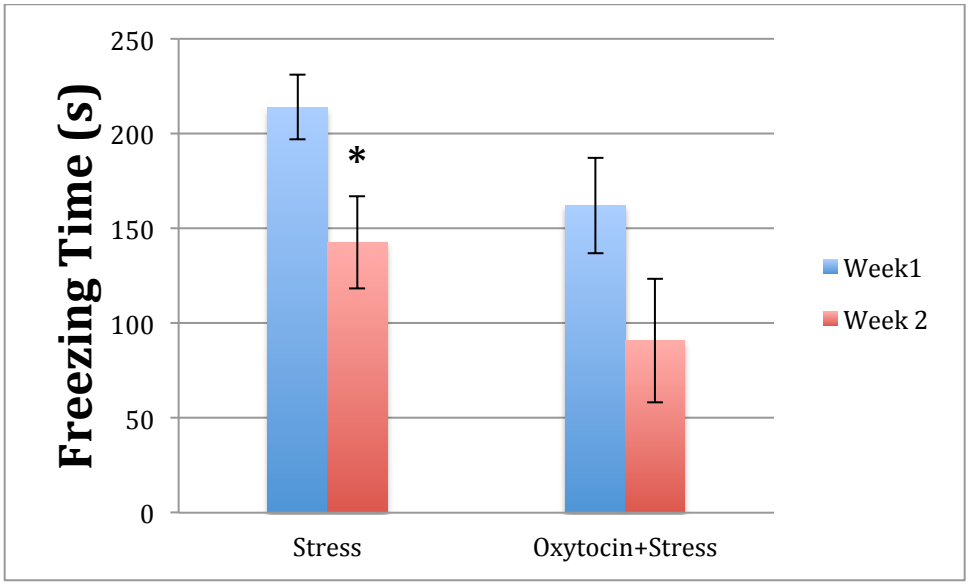


**Figure 11.** Comparison of freezing times between week 1 and week 2 in Stress and Oxytocin+Stress groups.

* p<0.05 two-tailed t-test

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