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Effects of oxytocin on background anxiety in rats with high or low baseline startle

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Abstract

Rationale—Oxytocin has antianxiety properties in humans and rodents. However, the antianxiety effects have been variable.

Objectives—To reduce variability and strengthen to the antianxiety effect of oxytocin in fear-potentiated startle, two experiments were performed. First, different amounts of light-shock pairings were given to determine the optimal levels of cue-specific fear conditioning and non-predictable startle (background anxiety). Second, the antianxiety effects of oxytocin were examined in rats with high and low pre-fear conditioning baseline startle to determine if oxytocin differentially affects high and low trait anxiety rats.

Methods—Baseline pre-fear conditioning startle responses were first measured. Rats then received 1, 5 or 10 light-shock pairings. Fear-potentiated startle was then tested with two trial types: light-cued startle and non-cued startle trials. In the second experiment, rats fear conditioned with 10 light-shock pairings were administered either saline or oxytocin before a fear-potentiated startle test. Rats were categorized as low or high startlers by their pre-fear conditioning startle amplitude.

Results—Ten shock-pairings produced the largest non-cued startle responses (background anxiety), without increasing cue-specific fear-potentiated startle compared to 1 and 5 light-shock pairings. Cue-specific fear-potentiated startle was unaffected by oxytocin. Oxytocin reduced background anxiety only in rats with low pre-fear startle responses.

Conclusions—Oxytocin has population selective antianxiety effects on non-cued unpredictable threat, but only in rats with low pre-fear baseline startle responses. The low startle responses are reminiscent of humans with low startle responses and high trait anxiety.

Oxytocin has recently gained attention as a potential treatment for anxious behavior and anxiety disorder symptoms (Meyer-Lindenberg, Domes, Kirsch, and Heinrichs, 2011; Bakermans-Kranenburg and van IJzendoorn, 2013). In humans, oxytocin administration decreases anxiety (de Oliveira et al., 2012a; 2012b), reduces conditioned negative responses (Petrovic et al., 2008) and facilitates extinction recall (Acheson et al., 2013; Eckstein et al.,

2015). Acute oxytocin treatment reduces physiological responses to combat imagery prompts in Post-traumatic stress disorder patients (Pitman et al., 1993) while chronic oxytocin treatment reduces anxiety in male, but not female, generalized anxiety disorder patients (Feifel et al., 2011). In rodents, systemically administered oxytocin reduces anxiety behaviors in the open field test (Uvnäs-Moberg et al., 1994), decreases step-down passive avoidance latencies (De Oliveria et al., 2007), increases punished crossings in the four-plate test (Ring et al., 2006), improves avoidance learning in a strain of highly-emotional male rats (Uvnäs-Moberg et al., 1999), but may impair extinction of contextually conditioned fear (Eskandarian et al., 2013).

The startle response is also influenced by oxytocin. Enhancement of the acoustic startle response is a common symptom of anxiety disorders (Grillon and Baas, 2003) and is a well-studied translational measure of fear and anxiety (Davis, 1986). However, the effects of oxytocin on startle are inconsistent. High-anxiety rats display high startle amplitudes and low plasma oxytocin levels (Uvnäs-Moberg et al., 1999). Predator-odor induced enhancement of startle in high-anxious rats is reversed by oxytocin administration (Cohen et al., 2010). But, high doses of oxytocin enhance baseline acoustic startle in normal rats (King, 1985). Furthermore, potentiation of the startle by social isolation correlates with reduced OT-receptor expression in the lateral septum (Nair et al., 2005), whereas increased lateral septum OT-receptor expression increased freezing in social defeated mice (Guzman, et al., 2013). In addition, oxytocin deficient male mice show low baseline startle amplitudes (Winslow et al., 1999; Fergeson et al., 2000), and mice with a CaMKIV deletion have low cortical oxytocin levels and a depressed baseline startle (Shum et al., 2005). In humans, intranasal oxytocin administration increases startle responding during presentations of negative stimuli (Striepen et al., 2012), during presentation of conditioned stimuli that signal predictable (Acheson et al., 2013), but not unpredictable aversive events (Grillon et al., 2012). Collectively these results suggest that oxytocin plays a role in modulating startle, but whether it is anxiolytic or anxiogenic is unclear.

Work in our lab using a variant of the fear-potentiated startle paradigm demonstrated that low doses of peripherally administered oxytocin (0.01 and 0.1 µg/kg, s.c.) selectively reduced non-cued startle during a fear-potentiated startle test (Missig et al., 2010). Because specific cue-fear potentiated startle was not reduced by oxytocin compared to non-cued startle trials, we suggested that oxytocin selectively reduced startle to non-cued startle stimuli, but not startle stimuli cued by a conditioned fear stimulus (Missig et al., 2010; Ayers et al., 2011). We concluded that oxytocin selectively decreased anxiety during unpredictable threat (called background anxiety), but did not diminish signaled, predictable threat (called cue-specific fear-potentiated startle).

As work with this paradigm has continued, the effect of oxytocin on the reduction of background anxiety has become inconsistent. In different cohorts, oxytocin would sometimes significantly reduce background anxiety, but other times the reduction would at best result in a statistical trend. Therefore in the present experiments, we decided to see if we could optimize the amount of fear conditioning which then might produce more consistency in our background anxiety measure, allowing us to further explore the oxytocin effect. Secondly, we chose to analyze the data with individual differences in mind. Splitting rats

into high and low startle groups corresponds well with previous findings indicating that responses to oxytocin treatment may be influenced by an animal's trait or dispositional anxiety (Uvnäs-Moberg et al., 1999; Amico et al., 2004; Mantella et al., 2003; Slattery and Neumann, 2010). Numerous lines of evidence suggest that differences in baseline startle responding may reflect different levels of trait or dispositional anxiety (Uvnäs-Moberg et al., 1999; Blaszczyk et al., 2000; Plappert and Pilz, 2002; Lopez-Aumantell et al., 2009; Vicens-Costa et al., 2011; Yen et al., 2012; Waters et al., 2014). Therefore, in the present experiments trait anxiety, as measured by pre-training startle, was examined as a variable that affects oxytocin's effect on background anxiety.

Methods

Subjects

Subjects were 157 male Sprague-Dawley rats obtained from Charles River Breeders and weighing between 225–250g at the time of purchase. All subjects were pair-housed in polypropylene shoebox cages in a climate-controlled facility with a 7:00 am – 7:00 pm light/dark cycle and given access to food and water ad libitum. All experiments began one week following arrival and were performed between 8:00 am and 5:00 pm. All procedures were in accordance with the US National Institutes of Health *Guide for the Care and Use of Experimental Animals* and approved by the University of Delaware IACUC.

Apparatus

Eight identical SR Lab ventilated startle chambers with clear Plexiglas cylinders (San Diego Instruments, San Diego, CA) were used for all portions of startle testing. Three LED lights positioned on the right wall produced 2600 lux and served as the conditioned stimulus. A floor insert made of ten 4-mm diameter stainless steel rods placed 4 mm apart inside the Plexiglas cylinders delivered shocks to the feet. During all sessions a background white noise of 65 dB was played continuously. Rats were trained and tested in the same chambers.

Startle responses were transduced into voltages proportional to the startle reflex by a piezoelectric strain gauge attached to the base of the Plexiglas startle cage. The voltages were rectified, digitized and converted into arbitrary units. The average of 1 ms arbitrary unit samples over 100 ms starting at the onset of the noise stimulus was used as a measure of startle amplitude.

Experimental Design

The startle paradigm follows a basic design consisting of three days of startle acclimation/matching (Day 1–Day 3), one day of classical fear conditioning (Day 4), followed by a fear-potentiated startle test session (Day 5). The first three days of startle acclimation began with a 5-minute background white noise period followed by 30 presentations of startle stimuli ranging from 95dB, 105dB, or 115dB 50ms white noise bursts (10 of each) given in a predetermined pseudo-random order with a 15s inter-trial interval. These sessions served the triple role of acclimating the subjects to the experimental environment, sorting subjects into experimental groups with similar amplitudes of baseline startle, and to permit mean “Pre-Fear” startle scores to be constructed for each subject (see Data Analysis section). Rats with

similar Pre-Fear startle amplitudes were assigned to different experimental conditions so the various group Pre-Fear startle amplitudes were matched across conditions.

On the fourth day (Day 4), rats were classically fear conditioned. Training sessions consist of a 5-minute acclimation period followed by pairings of light with a foot shock. Each pairing consisted of a 3 second presentation of the light which co-terminated with the 500 ms (0.6 mA) foot shock; the inter-trial intervals ranged from 60 s to 180 s in a pseudo-random order. For experiment 1, training consisted of 1, 5, or 10 light-shock pairings during training. Experiment 2a and 2b utilized the 10 light-shock pairing procedure.

Testing for fear-potentiated startle and background anxiety began on Day 5 twenty-four hours after fear conditioning. This test consisted of a 5 minute acclimation period followed by 70 startle trials with 15s intervals. The first 10 trials consisted of 95 dB, 105 dB, or 115 dB noise bursts presented in a predetermined pseudo-random order to re-acclimate subjects to the startle stimuli. These were not utilized in any analyses. The following 60 trials consisted of 95 dB, 105 dB, or 115 dB noise bursts presented either in the dark or co-terminating with the 3s light CS presented in a pseudo-random order. For each of the noise intensities, 10 trials were presented in the absence of the light and 10 trials were presented in the presence of the light.

Oxytocin Administration

In experiments 2a and 2b, 0.1 µg/kg, sc Oxytocin (Bachem America, Torrence, CA, catalog number H-2510) was administered 30 minutes prior to startle testing. For each experiment a frozen stock solution of 10 µg/mL was diluted to the desired concentration and kept on ice. All injections were made subcutaneously in the scruff of the neck.

Data analysis

For each startle stimulus, the mean startle amplitude Pre-Fear startle scores represent the mean startle amplitudes of all trials over each subject's three days of acclimation sessions (Day 1–Day 3). Each subject's Pre-Fear score was used to match the animals into experimental groups with similar group mean startle amplitudes. For experiment 1 and 2 (Analysis 1) each condition consisted of rats with high, medium and low startle amplitudes, so that each group's mean startle score was similar to the other groups in the experiment. The second analyses in experiment 2 involved an additional post-hoc re-sorting of subjects into High and Low Pre-fear conditions using a median split within each drug condition (Saline & OT), resulting in 4 groups total (Low-Saline, Low-OT, High-Saline, High-OT).

During the background anxiety and cued fear-potentiated startle test, subjects received presentations of startle in the presence of the light CS+ (Light+Noise trials) or in its absence (Noise Alone). These 30 trials of each type were averaged to calculate a percent Fear-potentiated startle score (%FPS), which is the proportionate difference between (Light +Noise) and (Noise Alone) trials during testing [i.e. $\%FPS = ((\text{Light+Noise}) - (\text{Noise Alone})) / (\text{Noise Alone}) \times 100$]. Percent background anxiety (%BA) was calculated as the proportionate difference between the Noise Alone Trials during testing, and the Pre-fear scores [i.e. $\%BA = (\text{Noise Alone}) - (\text{Pre-fear}) / (\text{Pre-fear}) \times 100$]. Mixed-model and one-way

analysis of variance tests followed by post-hoc analyses were used to analyze the data in each of the experiments.

Results

Experiment 1: Determination of optimal number of light-shock pairings to induce fear-potentiated startle and background anxiety

Experiment 1 compared the effect of 1, 5 and 10 light+shock pairings during training on startle performance during testing. For this experiment a total of 48 subjects were sorted into 3 conditions, designated 1x ($n=12$), 5x ($n=24$), 10x ($n=12$). A mixed-model analysis of variance comparing Trial Type (Noise Alone, Light+Noise) by Shock Pairings (1x, 5x, 10x) revealed a significant main effect of Trial Type ($F(1,45)=103.497$, $p=0.000$), a main effect of Shock Pairings ($F(2, 45)=7.865$, $p=0.001$), and a Trial Type by Shock Pairing interaction ($F(2,45)=7.267$, $p=0.002$). A Tukey's Honestly Significant Post-hoc test revealed significant differences between the 1x and 5x shock pairings ($p=0.036$), and between the 1x and 10x shock pairings ($p=0.001$). The analyses indicate that 5x and 10x light-shock pairings produced greater startle in both noise alone (mean and standard deviation: $m_{5x}=65.33$ $sd_{5x}=45.623$; $m_{10x}=75.10$ $sd_{10x}=48.025$) and light-noise trials ($m_{5x}=115.36$ $sd_{5x}=62.681$; $m_{10x}=140.72$ $sd_{10x}=62.931$) compared to 1x pairing (Noise alone $m_{1x}=26.65$ $sd_{1x}=45.284$; light-noise $m_{1x}=55.21$ $sd_{1x}=19.633$) (Fig. 1a).

The effects of shock pairings on the cue-specific fear-potentiated startle (%FPS) and background anxiety (%BA) were examined next. A one-way analysis of variance of %FPS did not find a significant difference between the number of light-shock pairings ($F(2,45)=0.428$, $p=0.654$). However, a one-way analysis of variance examining %BA revealed a significant main effect of Shock Pairings ($F(2,45)=6.083$, $p=0.005$). A Tukey's honestly significant post-hoc test revealed a significant difference between the 1x and 10x training conditionings ($p=0.004$), but no difference between 1x and 5x ($p=0.346$) on %BA. Additionally, while the Tukey's test revealed no significant difference between 5x and 10x conditions on background anxiety, a more liberal least-significant difference (LSD) post-hoc test revealed a trend of $p=0.08$. Together, the analyses show that the %FPS was increased to a similar proportional level with 1x, 5x, or 10x light-shock pairings. In contrast, %BA was significantly increased by 10x light-shock pairings ($m_{1x}=11.12$, $sd_{1x}=47.656$; $m_{5x}=73.19$ $sd_{5x}=82.601$; $m_{10x}=141.65$ $sd_{10x}=135.907$). (Fig. 1c).

Experiment 2, Analysis 1: Effect of oxytocin on background anxiety and fear-potentiated startle in a large pool of subjects

Given the evidence from the previous experiment, a follow-up study utilized 109 subjects to determine whether oxytocin would alter the expression of background anxiety and fear-potentiated startle. This experiment's protocol was identical to Experiment 1 except for two parameters: all subjects received 10 stimulus pairings during fear-conditioning (Day 4), and all subjects received subcutaneous injections of either oxytocin (0.1 $\mu\text{g/kg}$) or the saline vehicle 30 minutes prior to background anxiety and fear-potentiated startle testing (Day 5).

Figures 2a–c display the results of this experiment. A mixed-model analysis of variance comparing Trial Type (Noise Alone vs. Light+Noise) by Drug (Oxytocin vs. Saline) revealed a significant main effect of trial ($F(1,107)=191.701$, $p=0.000$), but no main effect of drug ($F(1, 107)=1.322$, $p=0.253$), and no trial by drug interaction effect ($F(1,107)=0.154$, $p=0.695$). Furthermore, individual analysis of variance tests examining the effect of drug on percent fear-potentiated startle ($F(1, 107)=2.208$, $p=0.14$) and percent background anxiety revealed no effect, but a trend in the direction of greater background anxiety as more shocks were administered during fear conditioning ($F(1, 107)=2.696$, $p=0.10$).

Experiment 2, Analysis 2: Effect of oxytocin on background anxiety and fear-potentiated startle in subjects with high and low pre-fear startle

Although we were unable to replicate our previous findings (Missig et al., 2010; Ayers et al., 2011), the 0.1 µg/kg, s.c. dose of oxytocin appeared to produce a trend towards lower levels of background anxiety ($p=0.1$) in the direction of our earlier findings. One possible reason for this might be that individual differences might be a factor, where subjects vary in their pre-training startle levels (Pardon et al., 2002; Lopez-Aumantell et al., 2009; Vicens-Costa et al., 2011). We therefore explored the role of pre fear-conditioning startle amplitude by splitting the subjects into high and low startlers using each subject's Pre-Fear startle score to determine whether oxytocin differentially affected high or low startlers.

Pre-Fear scores from the 109 subjects were used to perform a median split within each the oxytocin and vehicle groups to obtain four groups: Low-Saline ($n=28$), Low-OT ($n=27$), High-Saline ($n=27$), High-OT ($n=27$). Individual analyses compared fear-potentiated startle and background anxiety between Low-Saline and Low-OT subjects and between High-Saline and High-OT subjects.

In Low Pre-Fear subjects a mixed model analysis of variance comparing trial (Noise Alone vs. Light+Noise) by drug (Saline vs. OT 0.1 µg) revealed a significant main effect of trial ($F(1,53)=134.824$, $p<0.001$), a significant main effect of drug ($F(1,53)=4.193$, $p=0.046$), but no significant interaction effect ($F(1,53)=0.069$, $p=0.794$). Using proportional startle scores, an independent samples t-test comparing %FPS between Low-Saline and Low-OT found no significant differences between groups ($t(53)=1.703$, $p=0.094$). However, an independent samples t-test comparing %BA between Low-Saline and Low-OT revealed a significant differences between saline and OT treated subjects ($t(53)=-2.215$, $p=0.031$). The data indicate that oxytocin significantly reduced background anxiety in Low Pre-Fear startlers (Figures 3a–c).

In High Pre-Fear subjects an analysis of variance comparing trial (Noise Alone, Light +Noise) by condition (Saline, OT 0.1µg) revealed a significant main effect of trial ($F(1,52)=83.775$, $p<0.001$) but no significant effect of drug ($F(1,52)=0.177$, $p=0.675$), and no significant interaction effect ($F(1,52)=0.103$, $p=0.75$). Individual independent samples t-tests comparing %FPS and %BA between High-Saline and High-OT subjects revealed no significant differences (%FPS: $t(52)=0.491$, $p=0.626$; %BA: $t(52)=-0.179$, $p=0.859$). The data show that oxytocin had no effect on High Pre-Fear startlers (Figures 4a–c).

Discussion

The findings of this study are two fold. First, the results from Experiment 1 indicate that additional training (10 pairings of light+shock) enhanced startle and expression of background anxiety during testing compared to 1 or 5 pairings. Interestingly, percent fear-potentiated startle scores were not affected by the number of light+shock pairings. Follow-up tests revealed that the differential effect on background anxiety and fear-potentiated startle was not due to variations in total time spent in the chamber during training with 1, 5 or 10 pairings (data not shown). Second, experiment 2 demonstrated that oxytocin's ability to reduce background anxiety was revealed only when the rats were sorted based on pre-training (Pre-Fear) startle performance. More specifically, subjects with low pre-fear startle showed reduced background anxiety following oxytocin treatment. High pre-fear subjects did not show a treatment effect.

The primary finding of Experiment 1, that the expression of background anxiety increases with additional training, provides important support to our original interpretation of the measure. The original investigations (Missig et al., 2010) revealed that oxytocin reduced a post-training enhancement of startle during noise-alone trials. Further study revealed that this post-training noise-alone enhancement was not standard contextual conditioning but rather it required conditioning to the light cue during training. Furthermore, oxytocin had no effect on startle when the light cue is absent from both training and testing (Missig et al., 2010). These findings suggested that during testing the light-cue had an influence on startle even during non-cue trials. We termed this enhancement of post-training noise alone trails "background anxiety" as a means of distinguishing it from other known types of fear (e.g., contextually conditioned fear). The results of Experiment 1 further support our initial hypothesis that background anxiety is an index of learned anxiety distinct from cue-specific fear-potentiated startle (Missig et al., 2010; Ayers et al., 2011), and suggest that different mechanisms might be responsible for acquiring and modulating background anxiety and cue-specific fear-potentiated startle – possibly those related to the predictive properties the cue acquires during conditioning. Additionally, given the findings that 10 light+shock pairings evoked more robust expression of background anxiety, experiment 2 utilized the 10 light+shock pairing training protocol.

The initial findings of Experiment 2, that pre-testing oxytocin does not affect background anxiety following 10 pairings of light+shock during training when all rats are included in the data analyses, appear to conflict with our previous results (Missig et al., 2010; Ayers et al., 2011). This could be due to extraneous factors such as different cohorts from the breeder, cohorts having different levels of anxiety because of housing conditions, seasonal variation in stress, and different experimenters having dissimilar effects on stress and behavior. Animals exposed to psychogenic stressors (Cohen and Zohar, 2004) and fear-conditioning (Miles et al., 2011) paradigms show varied behavioral phenotypes, which may reflect subpopulations within a cohort that respond differently to stressful/aversive events. This evidence corresponds well with experimental findings indicating that responses to oxytocin treatment may relate to an animal's dispositional anxiety (Uvnäs-Moberg et al., 1999; Slattery and Neumann, 2010) or stress reactivity (Lopez-Aumantell et al., 2009).

The median split analysis we used revealed that pre-testing oxytocin administration reduced background anxiety in subjects with low pre-fear startle, but not subjects with high pre-fear startle. Additionally, oxytocin had no effect on fear-potentiated startle in any of the groups. These results are in good agreement with evidence that subjects high in stress reactivity and anxiety behavior show depressed baseline startle responses (Uvnäs-Möberg et al., 1999; Lopez-Aumantell et al., 2009; Vicens-Costa et al., 2011; Yen et al., 2012). One idea is that low levels of startle, such as our Pre-Fear measure, may reflect high trait anxiety in rats (Uvnäs-Moberg et al., 1999; Slattery and Neumann, 2010) and distress (high threat vigilance) in human anxiety and depressive disorders (Waters, et al., 2014). Low startle responses might be a phenotype for high trait anxiety rats and humans with distress disorders (unipolar depressive disorders, dysthymia, generalized anxiety disorder, post-traumatic stress disorder).

Although not investigated in our study, high and low Pre-Fear startle and the response to oxytocin may be related mechanisms of the HPA-axis. High levels of circulating glucocorticoids are associated with enhancements of the acoustic startle response (Servatius et al., 1994; Uvnäs-Möberg et al., 1999; Cohen et al., 2008; Cohen et al., 2010). Oxytocin has been shown to regulate the HPA-axis and levels of circulating glucocorticoids (Windle et al., 1997; Petersson et al., 1999; De Oliveria et al., 2007; Cohen et al., 2010). PTSD patients display both abnormal HPA-axis activity (Yehuda, 2002) and abnormalities in startle responding (Grillon et al., 2009; Jovanovic et al., 2009; Norrholm et al., 2011). Similarly, alterations in adrenergic signaling have been shown to affect startle responding in rodents (Davis, 1979; Davis, 1986; Pardon et al., 2002; Toth et al., 2013; Park et al., 2013). Peripherally administered oxytocin is known to decrease heart rate and blood pressure (Petersson et al., 1996; Gimpl and Fahrenholz, 2001; Holst et al., 2002) and high levels of circulating oxytocin correlate with low plasma norepinephrine (Grewen and Light, 2011). It is therefore possible that our group differences in Pre-Fear startle and the background anxiety response to oxytocin treatment may relate to variations in action of a number of hormonal systems during threat.

One caveat, however, is that care should be taken to avoid blindly equating low startle with an anxious or distress phenotype since the opposite relationship is often found. For example, Plappert and Pilz (2002) found that mice bred for high anxiety behavior in the light/dark box, open field, and spontaneous locomotor tests, show greater acoustic startle and stimulus sensitization. Blaszczyk et al., (2000) bred mice for high or low stress-induced analgesia found that high analgesic mice show higher acoustic startle and more anxious behavior in the open field. Interestingly, mice deficient in oxytocin have decreased baseline startle amplitudes (Winslow et al., 1999; Fergusson et al., 2000; Shum et al., 2005), though this does not necessarily relate to an anxious behavioral phenotype. Furthermore, oxytocin administration does not consistently reduce anxiety behavior and may actually increase startle to threatening stimuli in healthy humans (Grillon et al., 2012; Striepens et al., 2012; however, see Ellenbogen et al., 2014).

Although we have only found oxytocin to decrease acoustic startle in rats, recent findings in humans have reported discrepant effects of oxytocin on acoustic eye-blink startle where oxytocin was shown to increase, decrease, or have no effect on acoustic startle during

presentation of emotionally-laden stimuli (Grillon et al., 2012; Striepens et al, 2012; Ellenbogen, et al, 2014). While it is unclear why these studies found conflicting results, individual differences in the startle reflex may contribute to the lack of agreement. A recent study reported that baseline startle and startle during a threatening context are influenced differentially in fear and distress disordered adolescents (Waters et al., 2014). Individuals with principal fear disorders (specific phobia; social phobia) had higher startle during baseline, threatening contexts and specific danger, whereas those with distress disorders (unipolar depressive disorders, dysthymia, generalized anxiety disorder; post-traumatic stress disorder) had lower startle at baseline and in a threatening context. Extrapolating to our study, rats that displayed lower pre-fear (baseline) startle might be more distressed than those with higher pre-fear startle. Oxytocin might have selective effects on distressed animals during unpredictable threat.

Overall our findings suggest that the pattern of startle responses during baseline and different threats affects the efficacy of oxytocin in reducing anxiety. If this relationship is correct, it might prove useful in designing treatments for human neuropsychiatric disorders characterized by trait anxiety and distress, particularly since results of clinical studies testing oxytocin have been inconclusive (Young and Barrett, 2015). Further investigation is needed to reveal whether the pharmacological basis of oxytocin's reduction of background anxiety is related to genotype and phenotype.

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Figure 1.

The effects of 1, 5 or 10 light-shock pairings on fear-potentiated startle and background anxiety. 5 and 10 light+shock pairings during training increased startle compared to 1 pairing (Figure 1a.). The percent of fear-potentiated startle did not differ between 1, 5 or 10 pairings (Figure 1b.). Percent of background anxiety was significantly increased with 10 pairings compared to 1 pairing, $p < 0.004$ (denoted by * in Figure 1c.).

Figure 2.

Lack of an oxytocin antianxiety effect when all of the animals were included in the analysis. Oxytocin did not affect startle (Figure 2a.), the percent of fear-potentiated startle (Figure 2b.), or percent of background anxiety (Figure 2c) in subjects trained with 10 pairings of light+shock when all of the animals were included in the ANOVA.

Figure 3.

Oxytocin's antianxiety effect in rats with low Pre-Fear startle. Oxytocin had a main effect of reducing startle (Figure 3a.) in rats with low Pre-Fear startle responses ($p < 0.046$), denoted by the * in Figure 3a. Whereas the percent of cue-specific fear-potentiated startle was unaffected by oxytocin (Figure 3b), the percent of background anxiety was significantly reduced by oxytocin, $p < 0.03$ (denoted by * in Figure 3c.)

Figure 4.

Oxytocin did not produce an antianxiety effect in rats with high Pre-Fear startle response. Neither startle (Figure 4a.), percent of cue-specific fear-potentiated startle (Figure 4b.), nor percent of background anxiety (Figure 4c.) was affected by oxytocin in high startle Pre-Fear rats.