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14. ABSTRACT
The purpose of the grant is to test whether exogenous oxytocin acts as an antianxiety agent and whether social support facilitates its antianxiety effects in a fear-potentiated startle paradigm. Oxytocin given systemically (0.1 µg/kg, sc) effectively reduced background anxiety, but not specific cue-potentiated fear. This was found when oxytocin was given either before fear conditioning (acquisition), immediately after fear conditioning (consolidation), or before retrieval/expression of conditioned fear-potentiated startle. Social isolation for 3 weeks potentiated startle; this was reversed by oxytocin. Intracerebroventricular infusion of oxytocin only reduced background anxiety with a very large dose (20 µg), suggesting indirect action in brain. It is concluded that oxytocin has unique antianxiety properties that reduce background and social-isolation anxiety – anxiety states not directly related to cue-specific fear, but are sustained beyond the immediate threat. Oxytocin might be promising as a drug with novel benefits for patients with PTSD.

15. SUBJECT TERMS
fear; anxiety; PTSD; startle; social isolation

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INTRODUCTION

PTSD can be considered a disorder of affective memory where reminiscence of aversive events becomes exaggerated, uncontrollable and frightening. Fear during PTSD also becomes generalized where it is not confined to the trauma, but occurs in other situations or stimuli too. While classic antianxiety and antidepressant drugs have some efficacy for PTSD, newer medications via novel mechanisms are needed. Exogenous oxytocin, a nonapeptide found naturally in the brain and body, may have anti-anxiety effects in animals and humans, and therefore may be effective in interfering with acquisition and retention of aversive memory and promoting extinction. To test this hypothesis in an animal model of PTSD, fear-potentiated startle in male rats was employed. FPS in rats has face-validity for PTSD because a major hallmark symptom of PTSD in humans is exaggerated startle.

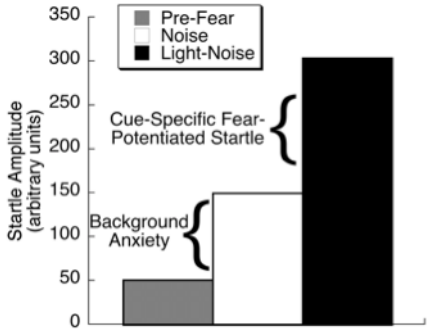
BODY

Completed work (Task 1; peripheral administration): Oxytocin Reduces Anxiety-Related Increases in Startle, But Not Cue Specific Fear-Potentiated Startle in Rats.

Publication (See Appendix 1): Oxytocin Reduces Background anxiety in a fear-potentiated startle paradigm (*Neuropsychopharmacology*, advance online publication, September 15, 2010, doi:10.1038/npp.2010.155).

This article describes the major finding for the project. It demonstrates that peripherally administered oxytocin does-dependently reduces background anxiety, but not cue-specific fear potentiated startle (Fig 1 for operational definitions of terms). Three experiments shown this effect on acquisition, consolidation and expression of fear-potentiated startle. Two additional experiments demonstrated that oxtyocin did not merely reduce the ability

Fig 1: Differences between trial types are used to define the effect son background anxiety and cue-specific fear-potentiated startle. The difference in startle between the Pre-Fear and Noise Trials is background anxiety.



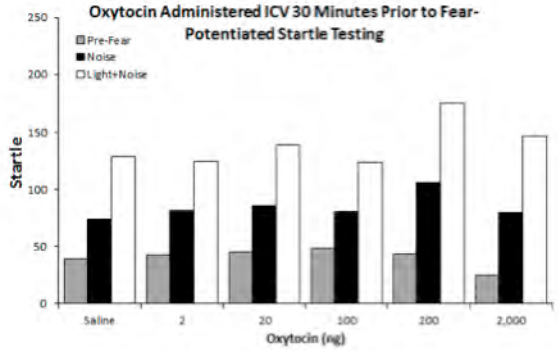
to startle and that oxtyocin's effect was not due to reduction of contextually conditioned fear. We concluded oxytocin has a novel antianxiety profile that targets background anxiety – an anxiety state not directly related to cue-specific or contextual fear, but sustained beyond the immediate threat.

Preliminary forms of this research were also presented at two meetings. The abstracts are in Appendix 2 and 3.

In Progress (Task 1, ICV administration): After much experimentation, we have found effects of oxytocin given intracerebroventricularly (ICV) on reducing background anxiety. Initially we

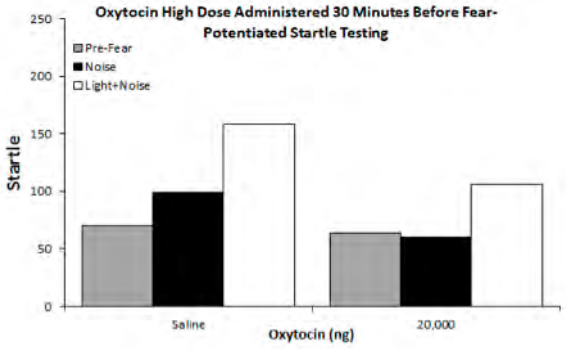
tested lower doses of oxytocin than were used for the peripheral studies because if it working in the brain effective doses should be lower. However this was not the case. Doses that worked peripherally, did not work when given ICV (Fig. 2). We therefore tried a very high

Fig. 2: ICV administration of oxytocin at doses that were effective peripherally does not reduces background anxiety.



dose that is 200 times higher than the most effective peripherally administered doses. As shown in Fig. 3, a dose of 20 µg, ICV effectively reduced background anxiety without diminishing cue-specific fear potentiated startle. Speculatively, oxytocin delivered ICV must return to the periphery and then become effective.

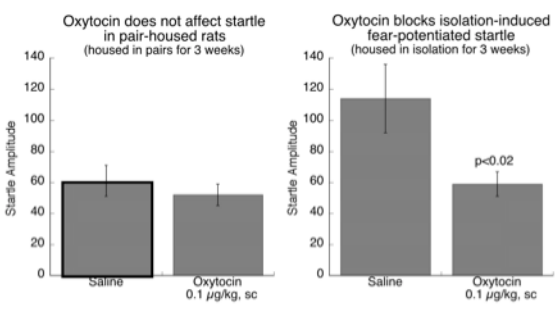
Fig. 3: A high dose of oxytocin (20 µg, ICV) significantly reduced background anxiety without affecting cue-specific fear potentiated startle.



An abstract of this work (Appendix 4) will be presented at the Society for Neuroscience meeting, November 2010. We are currently writing the manuscript for publication.

In Progress (Task 1; Social Support): The effects of oxytocin on buffering the effects of a lack of social support were tested. Comparison of the effects of oxytocin on rats housed in pairs versus isolated rats yielded significant differences on social isolation induced potentiation of startle. Pair-housed rats were tested for startle and then split into isolated or paired-housing for 3 weeks. Startle was tested again either with 0.1 µg/kg, sc oxytocin or saline. The results

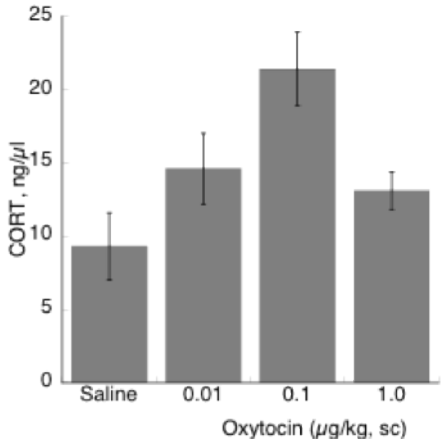
Fig. 4: Oxytocin has no effect on startle of pair-housed rats (left graph). Isolated-housing potentiates startle (right graph). Oxytocin significantly reduces the social isolation induced potentiated startle.



are shown in Fig. 4. This is a very exciting finding demonstrating oxytocin's antianxiety effects are not confined to fear-conditioning paradigm, but are quite robust and extend into social buffering. The experiment needs to be replicated before it is ready for publication.

In Progress (Task 2: Glucocorticoid measures): We have preliminary data on the effects of peripheral oxytocin on glucocorticoid levels in blood following the fear-potentiated startle test. 30 minutes after the fear-potentiated startle test, plasma blood was taken and the glucocorticoid levels were measured using an RIA for corticosterone (CORT). Fig. 5 shows the results. Whereas we thought oxytocin would decrease CORT because these rats displayed reduced background anxiety, the 0.1µg dose of oxytocin actually increased CORT levels. We do not understand the finding yet since we do not have a control group that just received oxytocin without fear conditioning and testing.

Fig. 5: Levels plasma CORT 30 minutes after a fear-potentiated startle test. 0.1 µg/kg oxytocin significantly reduced background anxiety (data not shown). Contrary to expectations, this dose significantly increased the blood levels of CORT compared to saline ($p<0.009$).



Training: A graduate and an undergraduate student were supported by the grant and have worked in collaboration on the completed studies. Each had no experience in behavioral pharmacology or fear-potentiated startle. The undergraduate (Galen Missig) began graduate school in Neuroscience this September at the University of Vermont. The graduate student (Luke Ayers) has presented some of the studies in a poster at the Society for Neuroscience meeting in Chicago last October. He will present the ICV data at this year's Society for Neuroscience meeting. Galen is first author on the Neuropsychopharmacology paper. Luke will be first author on the ICV paper that is being written now.

KEY RESEARCH ACCOMPLISHMENTS

- A new psychological target for antianxiety drugs is discovered – Background anxiety is an anxiety state not directly related to cue-specific or contextual fear, but sustained beyond the immediate threat.
- Systemically administered oxytocin is an effective antianxiety agent in male rats with unique properties of decreasing background anxiety but not cue-specific fear. This work has been published in *Neuropsychopharmacology*.
- Intracerebroventricularly administered oxytocin only reduces background anxiety when given in very high doses. This suggests that oxytocin is not working directly in brain.
- Oxytocin blocks the potentiation of startle induced by social isolation. Excitingly, oxytocin might buffer the detrimental effects of social isolation, a common problem in anxiety and depressive disorder patients.
- Taken together, it is concluded that oxytocin uniquely inhibits background and social isolation anxiety, while leaving fear to a specific fear stimulus intact.
- The research might have implications for oxytocin as a novel therapeutic treatment for PTSD, which there is a high degree of generalization of fear and anxiety.

KEY TRAINING ACCOMPLSHMENTS

- Graduate and undergraduate students (one each) have been trained in behavioral pharmacology using fear-potentiated startle as a paradigm for testing antianxiety drugs. The main undergraduate working on the project is now in graduate school in neuroscience.
- They have learned the proper procedures for conducting animal research.
- They have learned how to analyzes data, construct posters, and write abstracts and manuscripts.
- The students have learned presentation skills, and have presented the research at local and national (Society for Neuroscience meeting) research forums.

REPORTABLE OUTCOMES

1. Missig, G, Ayers, L.W., Schulkin, J. and Rosen, J.B. (2010). Oxytocin reduces background anxiety in a fear-potentiated startle paradigm. *Neuropsychopharmacology*, Advanced Online Publication, doi:10.1038/npp.2010.155.

2. Ayers, L.W., Missig, G., Schulkin, J. and Rosen, J.B. (2010). Systemic, but not intracereboventricular, administration of oxytocin results in an attenuation of background anxiety in fear-potentiated startle paradigm. Program No. 705.24. Society for Neuroscience: Neuroscience Meeting Planner, San Diego, CA. Online.

3. Ayers, L. W., Missig, G., Schulkin, J., Rosen, J.B. (2009). Oxytocin reduces anxiety-related increases in startle, but not cue-specific fear-potentiated startle in male rats: Relevance to PTSD. Program No. 841.17. 2009 Neuroscience Meeting Planner. Chicago, IL: Society for Neuroscience, 2009. Online.

4. Rosen, J.B., Missig, G., and Ayers, L.W. (2009). Oxytocin reduces anxiety-related increase in startle, but not cue-specific fear-potentiated startle in rats. Pressented at the Military Health Research forum, Kansas City, MO, September 1. Oral presentation #S3-11 and poster presentation #P19-13.

CONCLUSION

The project has been quite successful. I believe the article just published in *Neuropsychopharmacology* will be received very well and might lead people to investigating background anxiety as a therapeutic target. When I presented the preliminary results last year at the MHRF in Kansas City, there was a lot of interest in the work and they generated much discussion. Our more recent results from the intracerebroventricular administration of oxytocin should also produce a lot of interest and possibly controversy. They suggest that oxytocin's site of direct action is not the brain, but oxytocin may need to get back into the periphery to produce its actions. This may have implications for where the site of action is for the intranasal delivery of oxytocin in humans, which is thought to get into the brain but has not be definitively shown. Finally, the preliminary finding of oxytocin blocking the effects of social isolation is likely very important because it suggests oxytocin may induce social resiliency for patients of PTSD and other disorders which are co-morbid with unhealthy levels of social isolation.

Appendix 1:

Missig, G, Ayers, L.W., Schulkin, J. and Rosen, J.B. (2010). Oxytocin reduces background anxiety in a fear-potentiated startle paradigm. *Neuropsychopharmacology*, Advanced Online Publication, doi:10.1038/npp.2010.155.

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Oxytocin Reduces Background Anxiety in a Fear-Potentiated Startle Paradigm

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Oxytocin reportedly decreases anxious feelings in humans and may therefore have therapeutic value for anxiety disorders, such as post-traumatic stress disorder (PTSD). As PTSD patients have exaggerated startle responses, a fear-potentiated startle paradigm in rats may have face validity as an animal model to examine the efficacy of oxytocin in treating these symptoms. Oxytocin (0, 0.01, 0.1, or 1.0 μg, subcutaneously) was given either 30 min before fear conditioning, immediately after fear conditioning, or 30 min before fear-potentiated startle testing to assess its effects on acquisition, consolidation, and expression of conditioned fear, respectively. Startle both in the presence and absence of the fear-conditioned light was significantly diminished by oxytocin when administered at acquisition, consolidation, or expression. There was no specific effect of oxytocin on light fear-potentiated startle. In an additional experiment, oxytocin had no effects on acoustic startle without previous fear conditioning. Further, in a context-conditioned test, previous light-shock fear conditioning did not increase acoustic startle during testing when the fear-conditioned light was not presented. The data suggest that oxytocin did not diminish cue-specific conditioned nor contextually conditioned fear, but reduced background anxiety. This suggests that oxytocin has unique effects of decreasing background anxiety without affecting learning and memory of a specific traumatic event. Oxytocin may have antianxiety properties that are particularly germane to the hypervigilance and exaggerated startle typically seen in PTSD patients.

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Keywords: oxytocin; anxiety; fear; startle; PTSD

INTRODUCTION

Oxytocin has recently received considerable attention for its role in social behavior, and as a possible target for a number of psychiatric disorders, particularly, anxiety, post-partum depression, and autism (Carter, 2007; Heinrichs *et al*, 2009; Macdonald and Macdonald, 2010; Marazziti and Catena Dell'osso, 2008; Neumann, 2008). Oxytocin is a nonapeptide released in blood from the hypothalamo-neurohypophysial system and other peripheral organs, and in the brain within the hypothalamus, amygdala, bed nucleus of the stria terminals, brainstem, and other regions from neurons originating in the hypothalamic paraventricular and supraoptic nuclei (Gimpl and Fahrenholz, 2001; Kiss and Mikkelsen, 2005).

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Exogenous oxytocin has anxiolytic effects. Peripheral and central injections of oxytocin in rats and mice reduce anxiety in a number of tests when stress is high or induced (Rotzinger *et al*, 2010). Subcutaneous injections of oxytocin and oxytocin fragments in rats reduce retention of passive avoidance (Boccia and Baratti, 2000; de Oliveira *et al*, 2007; de Wied *et al*, 1987). Rats given low subcutaneous doses (1–4 μg/kg) of oxytocin spent more time in the center of an open field, similar to the behavior of rats given the anxiolytic benzodiazepine drug midazolam (Uvnäs-Moberg *et al*, 1994). A high-stress strain of Sprague–Dawley rats that typically perform poorly on conditioned avoidance showed significantly improved learning when given systemic oxytocin pretreatment (Uvnäs-Moberg *et al*, 2000).

In humans, exogenous intranasally administered oxytocin has anxiolytic effects in males (Domes *et al*, 2007; Heinrichs *et al*, 2003; Kirsch *et al*, 2005), diminishes aversive conditioning (Petrovic *et al*, 2008), and promotes emotional facial recognition (Di Simplicio *et al*, 2009; Fischer-Shofty *et al*, 2010) and memory (Savaskan *et al*, 2008). Oxytocin may have potential therapeutic use in social anxiety disorder (Guastella *et al*, 2009), autism (Andari *et al*,

2010), and possibly post-traumatic stress disorder (PTSD) (Pitman *et al*, 1993).

Acoustic startle as a measure of sensorimotor responsivity and anxiety (Braff *et al*, 2001; Davis *et al*, 2010; Swerdlow *et al*, 2008; Vaidyanathan *et al*, 2009) is also affected by oxytocin, but results are variable. High doses of oxytocin had no effect on startle (Feifel and Reza, 1999), but lower doses increased startle when tested in the dark phase of the day (King *et al*, 1985). Oxytocin null mice displayed low (Winslow *et al*, 2000) or normal (Caldwell *et al*, 2009) startle amplitudes. Oxytocin receptor knockout mice had normal acoustic startle (Lee *et al*, 2008). Oxytocin also did not affect pre-pulse inhibition of startle (PPI) by itself (Feifel and Reza, 1999), but disruption of PPI by phencyclidine was enhanced in oxytocin null mice (Caldwell *et al*, 2009), and oxytocin and a receptor agonist, WAY-267464, reversed the disruption in PPI induced by amphetamine and MK-801 in rats (Feifel and Reza, 1999; Ring *et al*, 2010). Highly emotional rats that have low plasma levels of oxytocin have increased startle (Uvnäs-Moberg *et al*, 1999). Similarly, Nair *et al* (2005) demonstrated that oxytocin receptor binding in the lateral septum was negatively correlated with the amplitude of startle potentiated by social isolation. Finally, humans homozygous for the G allele (GG) of a single-nucleotide polymorphism within intron 3 of the *OXTR* gene had lower levels of stress reactivity in anticipation of a startle stimulus than individuals with one or two copies of the A allele (AA and AG) polymorphism (Rodrigues *et al*, 2009). Together, these studies suggest that endogenous oxytocin and exogenously administered oxytocin modulate anxious states of rodents and humans.

Oxytocin has not been tested in fear-potentiated startle, which is often used as a measure of conditioned anticipatory anxiety and may model the hypervigilance and exaggerated startle responses typically seen in PTSD patients (Grillon and Morgan, 1999; Grillon *et al*, 2009b; Jovanovic *et al*, 2010, 2009; Morgan *et al*, 1995). One advantage of the fear-potentiated startle paradigm is that drug effects on fear or anxiety can usually be dissociated from motoric effects of drugs (Davis *et al*, 1993; Fendt *et al*, 2010; Joordens *et al*, 1998; Walker and Davis, 2002a). In the present experiments, oxytocin was administered systemically at various phases of learning, memory, and expression of fear to investigate its effects on acquisition, consolidation, and expression of conditioned fear. Our findings indicate a unique anxiolytic profile for oxytocin on startle and background anxiety, a state not directly related to cue-specific or contextually conditioned fear, but sustained beyond the immediate threat (Walker and Davis, 2002b).

MATERIALS AND METHODS

Animals

A total of 240 male Sprague–Dawley rats weighing between 225 and 250 g were obtained from Charles River Laboratories (Wilmington, MA). The rats were pair-housed in shoebox cages in a climate-controlled facility with a 0700–1900 hours light/dark cycle. Rats had free access to food and water. At 1 week after arrival, experiments were started and were performed between 0800 and 1600 hours. 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 training and testing. On one wall of each chamber, three LED lights in parallel produced 2600 lux and served as the conditioned stimulus (CS). A floor insert made of ten 4-mm diameter stainless steel tubes placed 4 mm apart inside the Plexiglas cylinder to deliver footshocks was used. Background white noise of 65 dB was continually played throughout all experimental sessions.

Experiment Design

Each experiment followed the basic paradigm: 3 days of startle acclimation/matching, 1 day of classical fear conditioning, and after a 96-h gap, a fear-potentiated startle test session. Deviations from this pattern are noted below in the Experiment sections.

Startle Acclimation/Matching

For the first 3 days of the experiment, rats were habituated to the chamber and presented with startle stimuli. For each daily session, there was a 5-min acclimation period followed by 30 trials of startle stimuli. The series of trials consisted of white noise bursts of 10 trials each of 95, 105, or 115 dB noise bursts presented in a predetermined pseudorandom pattern with a 15 s intertrial interval. On the third day of acclimation, the startle amplitudes were averaged for each rat and the mean startle score was used to sort the rats into matched groups with similar levels of startle. The rats were then rehoused and paired with a member of the same group.

Fear Conditioning

On the fourth day, the rats were classically fear conditioned to the light. Following a 5-min acclimation period, five pairings of 3 s of the light CS co-terminating with a 500 ms, 0.6 mA foot shock occurred. The intertrial intervals ranged from 60 to 180 s.

Fear-Potentiated Startle Testing

After a 96-h rest, the rats were tested for fear-potentiated startle. The testing consisted of 5 min of acclimation followed by 70 startle trials with 15 s intervals. The first 10 trials that consisted of 95 dB noise bursts were not used in any analyses. The next 60 trials consisted of 95, 105, or 115 dB noise bursts, with half presented either in the dark or co-terminating with the 3 s light CS. Thus, for each noise burst intensity, there were 10 trials in the dark and 10 trials co-terminating with the light. The trials were presented in a predetermined pseudorandom pattern.

Oxytocin Administration

Each group of rats was administered either 0, 0.01, 0.1, or 1.0 μg/ml/kg of oxytocin dissolved in saline (Bachem Americas, Torrance, CA, catalog number H-2510). The choice of doses was based on studies of de Wied *et al* (1987) and Boccia *et al* (1998). The choice of injections 30 min before the session was based on Ring *et al* (2006). A frozen stock solution of 10 μg/ml oxytocin was diluted before each experiment and maintained on ice. Injections were given subcutaneously at the scruff of the neck.

Experiment 1: Oxytocin During Acquisition

Injections were given 30 min before conditioning to examine the effect on acquisition of learned fear. Doses of 0.0, 0.01, 0.1, and 1.0 μg/kg oxytocin were tested with 12 rats in each condition for a total of 48 rats.

Experiment 2: Oxytocin During Consolidation

Injections were given 20 min after conditioning to determine the effect on fear consolidation. Again vehicle and the same three doses were tested with 12 rats in each condition.

Experiment 3: Oxytocin During Expression

Injections were given 30 min before fear-potentiated startle testing on the eighth day (96 h after acquisition) to test for the effect on expression of fear-potentiated startle. The same doses of oxytocin were tested with 12 rats per dose.

Experiment 4: Oxytocin on the Acoustic Startle Response Without Fear Conditioning

This experiment tested whether oxytocin suppressed the ability to startle. Acclimation and matching were performed similarly as previously described. On the fourth day, rats were not put into the testing chambers, nor were they conditioned (no lights, no shocks). On the eighth day, oxytocin was administered 30 min before acoustic startle testing. Instead of using a combination of Light + Noise and Noise-only trials, the 30 trials presented during acclimation was used. The same doses of oxytocin were tested with 12 rats per dose.

Experiment 5: Oxytocin on Context Fear-Potentiated Startle

In addition to fear conditioning to the explicit cue, conditioning also occurs to the context during cue-specific fear conditioning. Testing for contextually conditioned fear is typically conducted by returning the subject to the context without presentation of the explicit fear CS (Jacobs *et al*, 2010). To examine whether oxytocin influenced contextually conditioned fear-potentiated startle or not, the same 3 days of acclimation, group matching for startle response, and light-shock fear conditioning on the fourth day were performed as described above. After 96 h, rats were given saline or oxytocin, and 30 min later, instead of testing cue-specific light CS fear-potentiated startle, contextual fear was examined by presenting only Noise trials. Thus, instead of receiving a combination of 60 Light + Noise and Noise trials, rats received 60 Noise trials in the same pseudorandom order as before. The same doses of oxytocin were tested with 12 rats per dose.

Data Analysis

For experiments 1 through 3, three startle scores were used for the statistical analyses: Pre-Fear startle, Noise, and Light + Noise. Startle amplitudes of each rat induced by the 95, 105, and 115 dB noise bursts (30 trials) from the last (third) acclimation session were averaged to obtain a single score of Pre-Fear startle. The same was done for the 30 Noise and 30 Noise + Light trials in the fear-potentiated startle test for Noise and Light + Noise scores, respectively. These scores were then used for statistical analyses.

The effect of oxytocin in the fear-potentiated startle test was analyzed by a mixed model ANOVA with a between-subject measure of dose (4 doses) and within-subject measure of fear-potentiated startle (Light + Noise vs Noise). *Post hoc* analysis of a main effect of dose on startle was performed with a Dunnett's test to compare the various doses of oxytocin to the vehicle (saline). Cue-specific conditioned fear was analyzed to two ways—using absolute fear-potentiated startle or proportional fear-potentiated startle scores. An absolute fear-potentiated startle score was computed by subtracting the average Noise startle amplitude from its average Light + Noise startle amplitude of each rat. A proportional fear-potentiated startle score for each rat was computed dividing the absolute fear-potentiated startle score by the average Noise startle amplitude. Analysis of proportional fear-potentiated startle was done to standardize the groups because fear-potentiated may be distorted by the baseline effects on oxytocin (Walker and Davis, 2002a). Dunnett's tests were used for these analyses.

A measure of change in startle amplitude after fear conditioning, which we call background anxiety, was also computed. Pre-Fear startle was compared with the Noise trials from the fear-potentiated startle test. Similar to the analysis of fear-potentiated startle described above, a mixed model ANOVA with a between-subject measure of dose and within-subject measure of background anxiety (Pre-Fear vs Noise) was performed. *Post hoc* analysis of a main effect of dose was performed with a Dunnett's test to compare the various doses of oxytocin to the vehicle (saline). A significant interaction effect was further analyzed with a Dunnett's test after the startle data was converted into background anxiety score (Noise minus Pre-Fear startle scores).

Experiments 4 and 5 did not test for Light + Noise startle. The Pre-Fear and Noise startle scores were statistically analyzed in a similar manner as the background anxiety measure of experiments 1–3. An α value of $p<0.05$ was considered a significant difference for all the analyses described above, but trends ($p<0.1$) are also presented in graphs.

RESULTS

The two important comparisons in this study are shown in Figure 1. Background anxiety is the comparison between Noise startle amplitude and Pre-Fear startle amplitude, and is the facilitating effect of cue-specific fear conditioning on

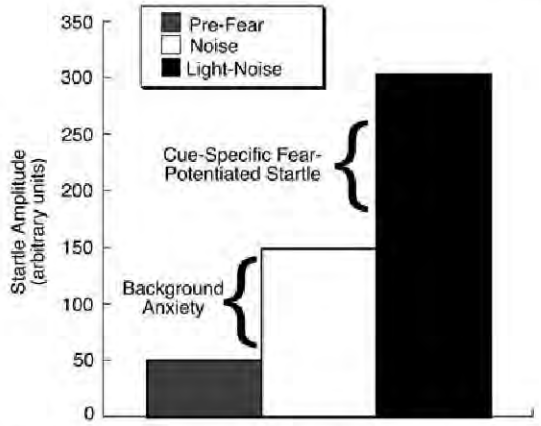

Figure 1 Sample startle responses. Startle from three different trial types were used to analyze the effects of oxytocin. Background anxiety is the increase in startle amplitude in the Noise trials during the fear-potentiated startle test compared with startle amplitude during the last acclimation session (Pre-Fear startle). Cue-specific fear-potentiated startle is the increase is startle amplitude in the Light + Noise trials compared with startle amplitude in the Noise trials during the fear-potentiated startle test.

Noise trials in the fear-potentiated startle test. Cue-specific fear-potentiated startle is the increase in Light + Noise startle amplitude compared with Noise startle amplitude due to the Light + footshock fear conditioning.

In general, regardless of when oxytocin was administered (ie, acquisition, consolidation or expression), it had similar effects on background anxiety and cue-specific fear-potentiated startle, but the effects were statistically most robust when oxytocin was administered 30 min before acquisition session or the fear-potentiated startle test. Oxytocin dose dependently diminished background anxiety and acoustic startle both in the presence and absence of light, but had no specific effect on cue-specific fear-potentiated startle.

Experiment 1: Oxytocin Effects on Acquisition

There was a significant main effect of cue-specific fear-potentiated startle (Light + Noise trials different from Noise trials, $F_{1,44} = 106.1$, $p < 0.0001$) and a trend for a main effect of oxytocin dose on startle amplitude ($F_{3,44} = 2.33$, $p < 0.088$). A Dunnett's test revealed a significant reduction in acoustic startle by 0.1 μg oxytocin compared with saline ($p < 0.034$, Figure 2a). There was no interaction effect indicating that oxytocin did not affect cue-specific fear-potentiated startle using absolute fear-potentiated startle scores. This was supported using proportional fear-potentiated startle scores (Figure 2b). Background anxiety was only marginally reduced by oxytocin. A mixed model ANOVA revealed a main effect of an increase in startle in Noise trials compared with Pre-Fear trials ($F_{1,44} = 27.0$, $p < 0.0001$). A Dunnett's test showed that there was a trend for the 0.1 μg dose of oxytocin to diminish background anxiety compared with saline ($p = 0.064$, Figure 2c).

Experiment 2: Oxytocin Effects on Consolidation

Similar to oxytocin given before acquisition, there was a significant within-measure main effect of fear-potentiated startle ($F_{1,44} = 147.8$, $p < 0.0001$; Figure 3a). There was a trend for a between-measure main effect of oxytocin on startle amplitude ($F_{3,44} = 2.81$, $p < 0.092$) and a Dunnett's test suggests this is because of reduced startle with 0.1 μg oxytocin compared with saline ($p < 0.046$, Figure 3a). There was a significant interaction effect ($F_{3,44} = 3.06$, $p < 0.038$) suggesting an effect of oxytocin on cue-specific fear-potentiated startle using absolute fear-potentiated startle scores. However, a Dunnett's test using proportional fear-potentiated startle scores was not significant indicating oxytocin did not affect cue-specific fear-potentiated startle when the scores were standardized (Figure 3b). Testing for significance of background anxiety, there was a significant overall increase in Noise startle ($F_{1,44} = 173.2$, $p < 0.0001$), but no main effect of oxytocin dose on startle amplitude, nor an interaction. A Dunnett's test suggests there was a trend for a reduction in background anxiety with 0.1 μg oxytocin ($p < 0.08$).

Experiment 3: Oxytocin Effects on Expression

Scores were not obtained from one rat because of equipment malfunction. The effects of oxytocin given 30 min before the fear-potentiated startle test were similar to the effects on acquisition and consolidation. There was a significant main effect of fear-potentiated startle ($F_{1,43} = 129.18$, $p < 0.0001$) and a significant main effect of oxytocin dose on startle amplitude ($F_{3,43} = 3.07$, $p = 0.038$). Shown in Figure 4a, Dunnett's test revealed that the 0.01 μg dose of oxytocin significantly diminished startle ($p = 0.022$) and the 0.1 μg dose just missed significantly reducing startle ($p = 0.054$). There was no effect of oxytocin on fear-potentiated startle using either absolute or proportional scores of fear-potentiated startle (Figure 4b). Analyzing background anxiety, there was an overall increase in startle to Noise compared with Pre-Fear startle ($F_{1,43} = 23.93$, $p < 0.0001$). Oxytocin reduced background anxiety (Figure 4c). There was no main effect of oxytocin dose on startle amplitude, but there was significant interaction ($F_{3,43} = 3.14$, $p = 0.035$). A Dunnett's test on the interaction effect revealed that 0.1 μg oxytocin significantly reduced background anxiety compared with saline ($p = 0.022$), and the other two oxytocin doses displayed a trend for reducing background anxiety (0.001 μg, $p = 0.094$; 1.0 μg, $p = 0.054$).

Experiment 4: Oxytocin does not Reduce the Ability to Startle

The previous experiments demonstrated that oxytocin reduces acoustic startle both in the absence and presence of the fear conditioned stimulus. While we are calling this a reduction in background anxiety, an alternative explanation is that oxytocin simply interferes with the ability to startle or respond to the acoustic stimulus. To test whether oxytocin is merely reducing the startle response, rats were not fear conditioned, but tested for startle amplitude with or without oxytocin. Within-subject comparisons were made between startle before receiving oxytocin and 30 min after oxytocin administration (Figure 5a). There were no effects of any dose of oxytocin on startle amplitude. Thus, oxytocin may not merely reduce the ability to startle, but seems to reduce startle subsequent to fear conditioning.

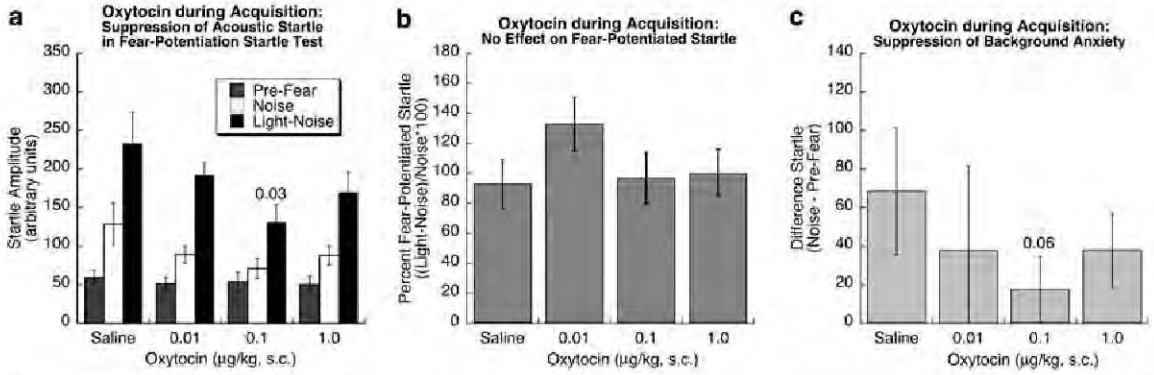

Figure 2 Effect of oxytocin administered before the acquisition phase. (a) Mean startle amplitudes of the three different trial types. The 0.03 above the Noise and Light + Noise startle scores is the *p*-value of the difference in startle between saline and 0.1 µg oxtyocin. No other comparisons approached statistical significance. (b) Proportional fear-potentiated startle scores. There were no statistical differences between any dose of oxytocin and saline. (c) Background anxiety scores. The 0.06 is the *p*-value of the difference in background anxiety startle scores between saline and 0.1 µg oxytocin. No other comparisons approached statistical significance.

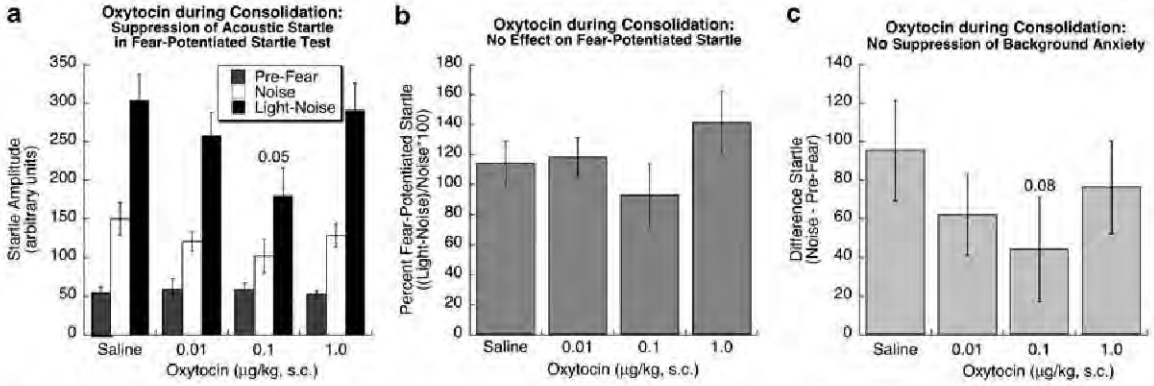

Figure 3 Effect of oxytocin administered in the consolidation phase. (a) Mean startle amplitudes of the three different trial types. The 0.05 above the Noise and Light + Noise startle scores is the *p*-value of the difference in startle between saline and 0.1 µg oxytocin. No other comparisons approached statistical significance. (b) Proportional fear-potentiated startle scores. There were no statistical differences between any dose of oxytocin and saline. (c) Background anxiety scores. There were no statistical differences between any dose of oxytocin and saline, but the 0.1 µg dose approached significance ($p < 0.08$).

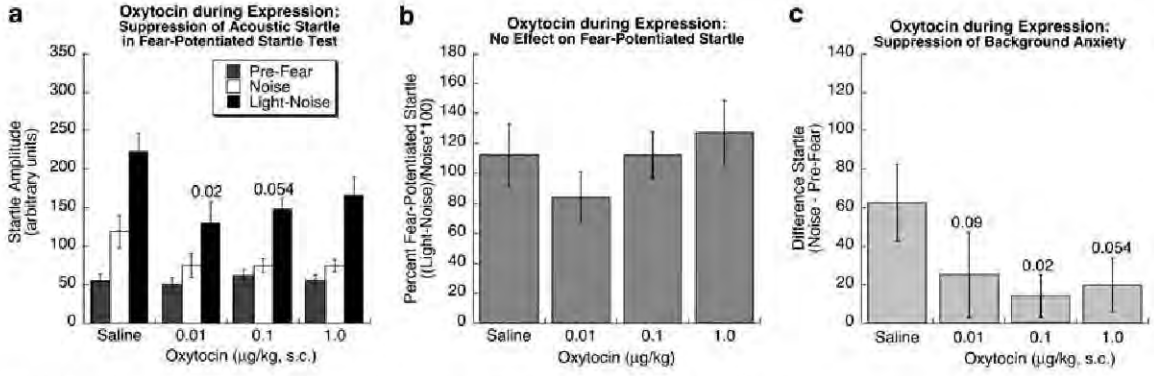

Figure 4 Effect of oxytocin administered before the fear-potentiated startle expression test. (a) Mean startle amplitudes of the three different trial types. The 0.02, and 0.054 above the Noise and Light + Noise startle scores are the respective *p*-values of the differences in startle between saline and the 0.01 and 0.1 µg doses of oxytocin. (b) Proportional fear-potentiated startle scores. There were no statistical differences between any dose of oxytocin and saline. (c) Background anxiety scores. The 0.09, 0.02, and 0.054 are the *p*-values of the differences in background anxiety startle scores between saline and the 0.01, 0.1, and 1.0 µg doses of oxytocin, respectively.

It is possible, however, that the lack of an effect of oxytocin on startle amplitude was because startle levels were very low in this experiment, and oxytocin may be more effective in reducing high levels of startle like those generated in experiments 1 through 3 following fear conditioning. We therefore reanalyzed the data of experiment 4

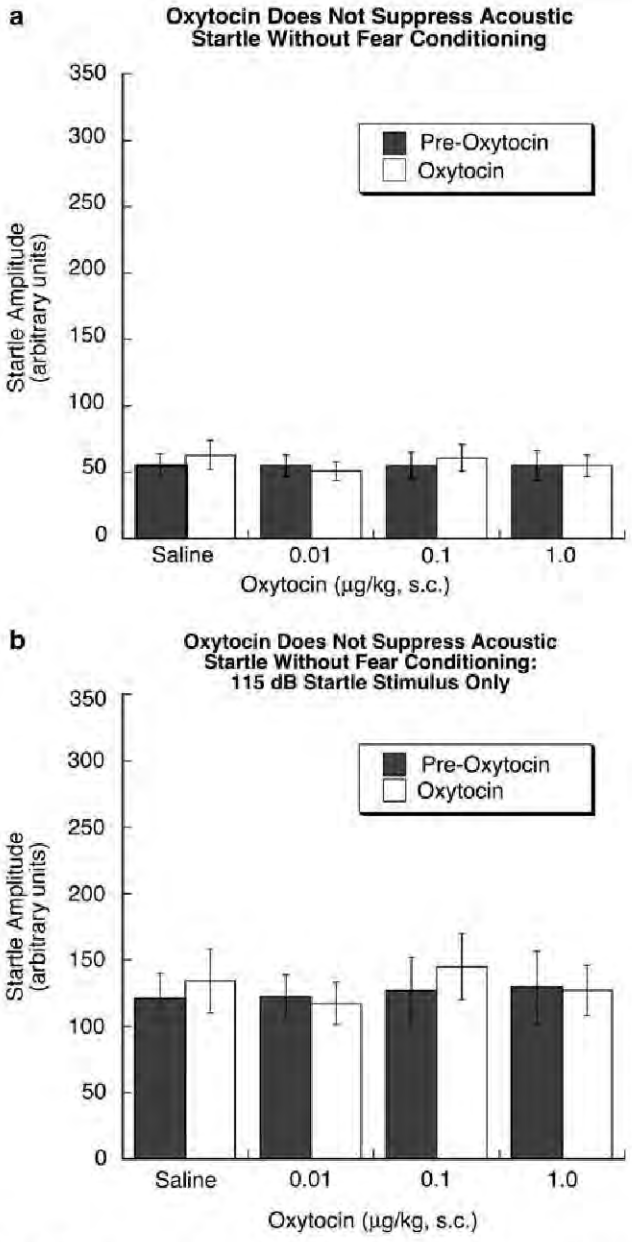

Figure 5 No effect of oxytocin on acoustic startle in rats that were not fear conditioned. (a) Startle amplitudes averaged from the 95, 105, and 115 dB startle stimuli. (b) Startle amplitudes from the 115 dB startle stimulus only.

using startle amplitudes induced by the three startle stimulus intensities, 95, 105, and 115 dB noise bursts, individually. There were no effects of oxytocin on startle elicited at any of these intensities. The mean pre-oxytocin and oxytocin startle amplitudes of the saline group induced by the 115 dB noise burst were 134 and 145, respectively (Figure 5b). These amplitudes are similar to the mean of the combined 95, 105, and 115 dB induced startle amplitudes of the Noise trials in the saline groups after fear conditioning in experiments 1 through 3, in which the startle amplitude means ranged from 119 to 150 startle units. Therefore, because similar levels of startle amplitude were reduced by oxytocin following fear conditioning, but not affected by oxytocin without previous fear conditioning, it is likely that

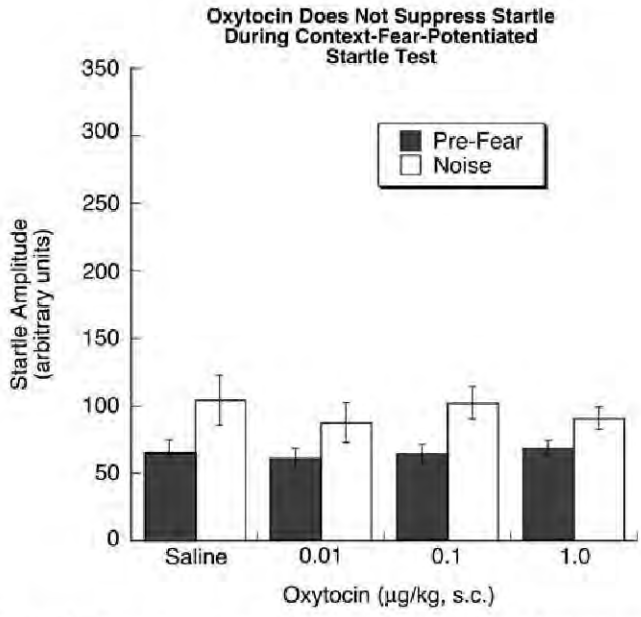

Figure 6 Test of contextually conditioned fear. No effect of oxytocin on acoustic startle in fear-conditioned rats not presented with the light CS during startle testing. There was a significant increase in startle in the Noise test compared with the Pre-Fear test in all the groups, indicating contextually conditioned fear. There were no differences in startle between the saline and oxytocin groups.

oxytocin reduces background anxiety and not the ability to startle.

Experiment 5: Oxytocin does not Reduce Contextually Conditioned Fear

Whereas we suggest that oxytocin is reducing background anxiety, it is possible that oxytocin interferes with conditioned contextual fear instead. To test this explanation, rats were tested for Pre-Fear acoustic startle amplitude, fear conditioned to the light, but then tested for startle without presenting the fear-conditioned light. Thus, if oxytocin decreased startle in the test without ever presenting the fear CS, it would indicate that oxytocin reduced contextually conditioned fear. There was a significant main effect of an increase in startle after fear conditioning ($F_{1,44} = 47.62$, $p < 0.0001$), but no significant main effect of oxytocin at any dose, nor an interaction effect (Figure 6). The results indicate that contextually conditioned fear was produced, but oxytocin did not reduce this conditioned fear as measured by startle amplitude, and suggest that the effects of oxytocin on startle in experiments 1 through 3 were due to its effects on some kind of background anxiety that is different from contextually conditioned fear.

DISCUSSION

The results of the present experiments indicate that oxytocin has unique effects on startle as measured in a fear-potentiated startle paradigm. Oxytocin did not have specific effects on cue-specific conditioned fear-potentiated startle, which is different from the cue-specific reduction of

fear-potentiated in rodents and monkeys by antianxiety drugs such as diazepam and buspirone (Davis, 1979; Joordens *et al*, 1998; Kehne *et al*, 1988; Risbrough *et al*, 2003; Winslow *et al*, 2007). Oxytocin, however, had a novel suppressant effect on startle, both in the presence and absence of the fear CS, but only if the fear CS was presented during the test. Furthermore, the increase in startle to Noise alone subsequent to fear conditioning (ie, background anxiety) was diminished by oxytocin. This unusual effect suggests that exogenous oxytocin acts as an anxiolytic agent, but does not diminish learned fear to a cue-specific or context CS. As discussed later, oxytocin may have particular therapeutic relevance for PTSD patients.

Subcutaneous oxytocin was shown to reduce acoustic startle given either during acquisition, consolidation, or expression of conditioned fear. Cue-specific fear-potentiated startle was not affected by oxytocin given at acquisition or expression (and with the proportional, but not absolute, fear-potentiated startle measure for consolidation), indicating that even though oxytocin diminished startle, there was no effect of oxytocin on cue-specific fear in the learning and expression phases. It is possible that oxytocin given during acquisition blunted nociception (Lundeberg *et al*, 1994) during fear conditioning, but there was no evidence of reduced cue-specific fear-potentiated startle in the acquisition experiment. Oxytocin also did not reduce the expression of acoustic startle in nonconditioned rats, nor contextually conditioned fear. These experiments indicate that oxytocin did not interfere with the ability to startle, nor the ability to learn cue-specific and contextually conditioned fear.

It is possible that oxytocin given before the fear-potentiated startle test reduced cue-specific fear. In this explanation, fear activated by the fear-conditioned light lingers through the 15-s intertrial intervals to enhance startle in the Noise-alone trials. Oxytocin might reduce cue-specific fear and consequently suppress the lingering fear throughout the intertrial interval and the Noise-alone trials. Although this possibility was not tested directly, de Jongh *et al* (2003) demonstrated that startle was not potentiated when the Noise was delivered 1–5 s after the offset of the light CS. This suggests that in our experiments the increase of startle in Noise-alone trials and its reduction by oxytocin were not due to cue-specific fear persisting into the Noise trials. Nonetheless, this explanation would need to be tested empirically before it is firmly rejected, possibly by testing whether there are lingering effects of the cue-specific fear CS in a novel context.

We hypothesize that oxytocin diminishes what we call background anxiety. This is an anxiety state not directly related to the cue-specific fear CS nor contextually conditioned fear cues, but is activated by the fear CS. This background state is evident during the testing of fear-potentiated startle by an increase in acoustic startle during Noise trials compared with acoustic startle in the Pre-Fear startle tests. Startle both in the absence and presence of the light fear CS was suppressed by oxytocin, but only if the light fear CS was presented during the fear-potentiated startle test session. Oxytocin given before acquisition or during consolidation could also diminish background anxiety without affecting learning and memory. The reduced background anxiety would then carryover to the test of expression to diminish startle when exogenous oxytocin was not present. Thus, oxytocin might be uniquely effective in reducing some type of background anxiety during a threatening situation that is not cue-specifically nor context-specifically conditioned.

A background anxiety-like phenomenon in a fear-potentiated startle paradigm has been observed before. Concomitant with intra-amygdala NMDA receptor blockade of cue-specific fear-potentiated startle, Walker and Davis (2002b) found a persistent increase in 'baseline' startle in both Noise and Light + Noise trials coinciding with the first light fear CS presentation. Background anxiety appears to be activated by cue-specific fear, but might be independent of it, likely because the two phenomena are subserved by different neural circuits (Walker and Davis, 2002b).

We conducted a test for contextually conditioned fear typically used in fear-conditioning experiments (Jacobs *et al*, 2010). Oxytocin had no effect on the contextual fear-conditioned increase in startle, which is different from the reduction of contextually conditioned fear in CRH receptor knockout mice using a shock-potentiated startle paradigm (Risbrough *et al*, 2009). Shock-potentiated startle, in which no explicit cues are paired with shock (Davis, 1989), enhances startle when wild-type animals are returned to the shock chamber (McNish *et al*, 1997; Richardson, 2000; Risbrough *et al*, 2009). Antagonism or knockout of CRH receptors reduces contextually conditioned shock-potentiated startle, but cue-specific fear-potentiated startle is not affected (Risbrough *et al*, 2009). Our conditioning protocol of contextual fear was different from the shock-potentiated startle paradigm, in that shock was paired with an explicit cue, relegating context conditioning to the background. In shock-potentiated startle, there is no cue-specific stimulus, and thus the context acts as a foreground stimulus similar to an explicit cue (Rescorla and Wagner, 1972). Whether oxytocin is also ineffective in a shock-potentiated startle paradigm with context as a foreground cue is a question for further research.

In our paradigm, oxytocin was effective at very low doses in the submicrogram range. Most studies of peripheral injections of oxytocin on anxiety tests (eg, elevated plus maze, light-dark box, open field, and acoustic startle) test doses in the milligram range (Feifel and Reza, 1999; King *et al*, 1985; Rotzinger *et al*, 2010). The submicrogram range effective in our studies is similar to those used in many intracerebroventricular and intracerebral infusion studies (Rotzinger *et al*, 2010). However, our doses are similar to those used in studies of peripherally administered oxytocin on inhibitory avoidance in rats (de Oliveira *et al*, 2007; de Wied *et al*, 1987; Kovacs *et al*, 1978) and post-training administration in mice (Boccia *et al*, 1998). Thus, startle appears to be as sensitive behavioral measure as passive avoidance for peripherally administered oxytocin, but does not answer the question of whether the site(s) of action are peripheral or central. Peripheral and central oxytocin systems are regulated differently, release very different amounts of oxytocin, and metabolize oxytocin at different rates, suggesting that the two systems are largely independent (Veening *et al*, 2010). We have preliminary data that oxytocin infused into the lateral ventricle in the same range of doses we administered subcutaneously might not reduce fear-potentiated startle or background anxiety (Ayers *et al*,

2010). In the periphery, oxytocin might possibly be acting by modulating glucocorticoid release at the adrenal glands (de Oliveira *et al*, 2007) or at the heart and vasculature to influence heart rate and blood pressure, as oxytocin receptors are located in these organs (Kiss and Mikkelsen, 2005). Clearly, much more research is needed before the sites of action and mechanisms of oxytocin on background anxiety are known.

The unique effects of oxytocin on startle in the fear-potentiated startle paradigm may have particular relevance for PTSD. Potentiation of startle in PTSD patients may be particularly sensitive to 'context fear' or 'contextualization' (Grillon, 2002; Liberzon and Sripada, 2008; Rougemont-Bücking *et al*, 2010), but not cued fear. The nature of this context fear in human studies is not clear—it may be a result of contextual fear conditioning, verbal instructions of the experiment, or increased fear/anxiety induced by the aversiveness of the experiments (Böcker *et al*, 2001, 2004; Grillon, 2002; Rougemont-Bücking *et al*, 2010). Context fear might be the same as what we call background anxiety, that is, 'fear-potentiated startle is riding on an already elevated baseline' (Grillon, 2002). In our case, the background anxiety is not contextually conditioned fear, and is likely analogous to the hypervigilance and sensitized emotional anticipation (Rosen and Schulkin, 1998) hypothesized to increase startle in the face of perceived threats accompanying patients with PTSD and panic disorder (Grillon *et al*, 1994; Grillon and Morgan, 1999; Grillon *et al*, 2009b; Morgan *et al*, 1995). In this regard, combat veterans with PTSD also display disruptions in PPI (Grillon *et al*, 1998, 1996), a nonlearned measure of sensorimotor gating (Braff *et al*, 2001), and oxytocin and an oxytocin receptor agonist reverse drug-induced disruption in PPI in rodents (Feifel and Reza, 1999; Ring *et al*, 2010). Therefore, oxytocin might specifically alleviate one or more physiopathologies of PTSD.

The effect of oxytocin on background anxiety in our fear-potentiated startle studies in rats is also reminiscent of the findings from some studies with anxiolytic and antidepressant drugs on context fear in humans, in which aprazolam, diazepam, oxazepam, and a 2-week treatment of citalopram reduce increased baseline startle, but not cue-specific fear-potentiated startle (Baas *et al*, 2002; Grillon *et al*, 2006, 2009a). This does not appear to be due to sedative effects of the drugs, but to a reduction in context fear (Grillon *et al*, 2006). Oxytocin similarly reduces increased background anxiety without diminishing cue-specific fear-potentiated startle, and does not appear to produce sedation, or at least, diminish the ability to startle. Testing of oxytocin in fear-potentiated startle in humans awaits future research.

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DISCLOSURE

The authors declare no conflict of interest.

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Appendix 2:

2010 Society for Neuroscience Abstract #705.24

Systemic, but not intracerebroventricular, administration of oxytocin results in an attenuation of background anxiety in a fear-potentiated startle paradigm

L. W. AYERS, G. MISSIG, J. SCHULKIN, J.B. ROSEN

Oxytocin is a compound long reported to have anxiolytic effects that may depend on an animal's state of anxiety. Recent work in our lab using the fear-potentiated startle paradigm has supported this claim; systemically administered oxytocin (0.01-1.0 μg oxytocin, s.c.) reduces background anxiety, yet specific conditioned fear is left intact. The effect was not due to oxytocin reducing the rats' ability to startle, nor in reducing contextual fear; rather it appears to relate to generalized anxiety that intermittent CS presentations produce. It remains to be determined if systemically administered oxytocin crosses the blood brain barrier to act in the brain directly, or whether its effects are initiated via interactions in the periphery. To address this question oxytocin was administered directly into the lateral ventricles of rats prior to testing fear potentiated startle. Eighty-eight male Sprague-Dawley rats were implanted with unilateral guide cannula aimed at the left lateral ventricle (ICV). Following recovery, each subject was acclimated to the startle apparatus and acoustic startle stimuli for 3 days. On the 4th day subjects were given standard Pavlovian fear conditioning; 5 pairings of a light and shock. On the 5th day, rats were sorted into four equal groups based on their startle response on the last acclimation day and then tested for fear-potentiated startle under the influence of oxytocin. ICV infusions of oxytocin (ranging from 2ng to 2000ng) were administered 30 min prior to receiving startle stimuli either in the presence or absence of the light. Remarkably, no dose of oxytocin had an effect on any measure of startle. To confirm that oxytocin administered ICV had behavioral effects, genital grooming after ICV administration was tested. Grooming bouts were significantly increased by 100ng and 1000ng oxytocin. Thus, the lack of effects of ICV oxytocin infusion on startle, together with the reduction in startle seen with systemic administration, suggests that oxytocin's effect of reducing background anxiety may be initiated in the periphery. These findings and future preclinical investigations into the mechanisms underlying oxytocin's reduction in background anxiety could lead to novel treatments for anxiety disorders, such as PTSD.

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Appendix 3:

2009 MHRF Oral presentation #S3-11 and poster presentation #P19-13

OXYTOCIN REDUCES ANXIETY-RELATED INCREASES IN STARTLE, BUT NOT CUE-SPECIFIC FEAR-POTENTIATED STARTLE IN RATS

Jeffrey B. Rosen, Galen Missig, and Luke W. Ayers

Oxytocin increases trustworthiness and well-being, while decreasing anxious feelings in men and women. Oxytocin, therefore, may have therapeutic value for anxiety disorders, like post-traumatic stress disorder (PTSD). To test this hypothesis, the effects of oxytocin were assessed on fear-potentiated startle in male rats. Because PTSD patients have exaggerated startle responses, fear potentiated startle in rats has face validity as an animal model to examine the effects of oxytocin on fear-exaggerated startle.

Methods: Fear-potentiated startle male Sprague-Dawley rats (225–250 g) from Charles River were housed in pairs. Startle was measured in a startle-sensitive apparatus. There were three phases of the fear-potentiated startle paradigm. Rats were first given a series of acoustic startle stimuli (95, 105, and 115 dB 50 ms white noise) on three consecutive days to determine their baseline startle amplitude. They then received Pavlovian fear conditioning of five pairings of a 3 s light co-terminating with a 500 ms, 0.6 mA footshock. Four days later, rats were tested for long-term memory of conditioned fear by delivering startle stimuli either in the presence or absence of the fear conditioned light. Fear-potentiated startle was defined as higher amplitude startle in the presence of the light compared to startle in its absence. Oxytocin (0, 0.01, 0.1, or 1.0 µg, s.c.) was administered 30 minutes before either fear conditioning, immediately after fear conditioning, or before fear potentiated startle testing to assess its effects on acquisition, consolidation, and expression of conditioned fear, respectively. Startle amplitude without fear conditioning. The effects of oxytocin also were assessed on acoustic startle without fear conditioning. Rats were given a random series of acoustic startle stimuli on three consecutive days to determine their baseline startle amplitude. Four days later rats received 0, 0.01, 0.1, or 1.0 µg, s.c. oxytocin 30 minutes before another series of acoustic startle stimuli. Differences in startle amplitude before oxytocin and during oxytocin were analyzed.

Results: Oxytocin had similar dose-dependent effects on startle during the fear-potentiated startle test when administered at any of the three phases (acquisition, consolidation, or fear expression). There were no specific effects on fear-potentiated startle. However, startle both in the presence and absence of the light was diminished by 0.1 µg of oxytocin, regardless of when oxytocin was administered. This indicated that acoustic startle, but not fear-potentiated startle, was diminished by oxytocin. To examine whether oxytocin interacted with fear conditioning, oxytocin was tested on startle of rats without prior fear conditioning. There was no effect of oxytocin at any of the doses tested.

Conclusions and Impact: Peripheral administration of oxytocin did not diminish cue-specific conditioned fear, but reduced nonspecific anxiety. The findings suggest oxytocin has unique effects of decreasing generalized anxiety without affecting learning and memory of a specific traumatic event. Oxytocin may have anti-anxiety properties that are particularly germane to the generalization of trauma typically seen in PTSD patients.

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Appendix 4:

2009 Society for Neuroscience Abstract # 841.17

Oxytocin reduces anxiety-related increases in startle, but not cue-specific fear-potentiated startle in male rats: Relevance to PTSD.

Luke W. Ayers, Galen Missig, Jay Schulkin and Jeffrey B. Rosen

Oxytocin reportedly decreases anxious feelings in humans and may therefore have therapeutic value for anxiety disorders, like post-traumatic stress disorder (PTSD). Since PTSD patients have exaggerated startle responses, a fear-potentiated startle paradigm in rats may have face validity as an animal model to examine the efficacy of oxytocin in treating these symptoms. Male Sprague-Dawley rats were used in a 3-phase fear-potentiated startle paradigm. Rats were first given a series of acoustic startle stimuli (95, 105 and 115 dB, 50 ms duration) on 3 consecutive days to determine baseline startle amplitude. They then received Pavlovian fear conditioning of five pairings of a 3 s light co-terminating with a 500 ms, 0.6mA footshock. Four days later, rats were tested for conditioned fear by delivering startle stimuli either in the presence or absence of the fear conditioned light. Fear-potentiated startle was defined as higher amplitude startle in the presence of the light compared to startle in its absence. Oxytocin (0, 0.01, 0.1, or 1.0 µg, s.c.) was given 30 min before fear conditioning, immediately after fear conditioning, or 30 min before fear-potentiated startle testing to assess its effects on acquisition, consolidation and expression of conditioned fear, respectively. Startle both in the presence and absence of the light was significantly diminished by oxytocin (0.1 µg/kg) when administered at any of the three phases (acquisition, consolidation, or fear expression). There was no specific effect on fear-potentiated startle. Oxytocin also had no effects on acoustic startle during testing without previous fear conditioning. Further, in a context-conditioned test, previous light-shock fear conditioning did not increase acoustic startle during testing when the light was not presented. The data suggest that oxytocin did not diminish cue-specific conditioned fear, nor contextual fear, but reduced nonspecific anxiety. This suggests that oxytocin has unique effects of decreasing generalized anxiety without affecting learning and memory of a specific traumatic event. Oxytocin may have antianxiety properties that are particularly germane to the generalized hypervigilance and exaggerated startle typically seen in PTSD patients.

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