

AD_____

AWARD NUMBER: W81XWH-08-1-0182

TITLE: Oxytocin and Social Support as Synergistic Inhibitors of Aversive Fear Conditioning and Fear-Potentiated Startle in Male Rats

PRINCIPAL INVESTIGATOR: Jeffrey B. Rosen, Ph.D.

CONTRACTING ORGANIZATION: University of Delaware
Newark, DE 19716

REPORT DATE: September 2009

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE 1 September 2009		2. REPORT TYPE Annual		3. DATES COVERED 1 Mar 2008 – 31 Aug 2009	
4. TITLE AND SUBTITLE Oxytocin and Social Support as Synergistic Inhibitors of Aversive Fear Conditioning and Fear-Potentiated Startle in Male Rats				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-08-1-0182	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Jeffrey B. Rosen, Ph.D. E-Mail: jrosen@udel.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Delaware Newark, DE 19716				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The purpose of the grant is to test whether exogenous oxytocin acts as an antianxiety agent and whether social support to facilitate its antianxiety effects in a fear-potentiated startle paradigm. Oxytocin given systemically (0.1 µg/kg, subcutaneous) effectively reduced background or generalized 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. Initial tests of social support (pair-housed vs. isolated rats) did not alter the antianxiety effect of oxytocin. Additional experiments testing the effects of intraventricular or amygdala infusion of oxytocin are inconclusive, but these experiments are still ongoing. The generalized antianxiety effect of peripherally administered oxytocin, but not a specific effect on conditioned fear, is novel and suggests that oxytocin may have unique antianxiety properties.					
15. SUBJECT TERMS fear; anxiety; PTSD; startle					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 12	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
Introduction.....	1
Body.....	1
Key Research Accomplishments.....	5
Reportable Outcomes.....	6
Conclusion.....	7
Appendices.....	8

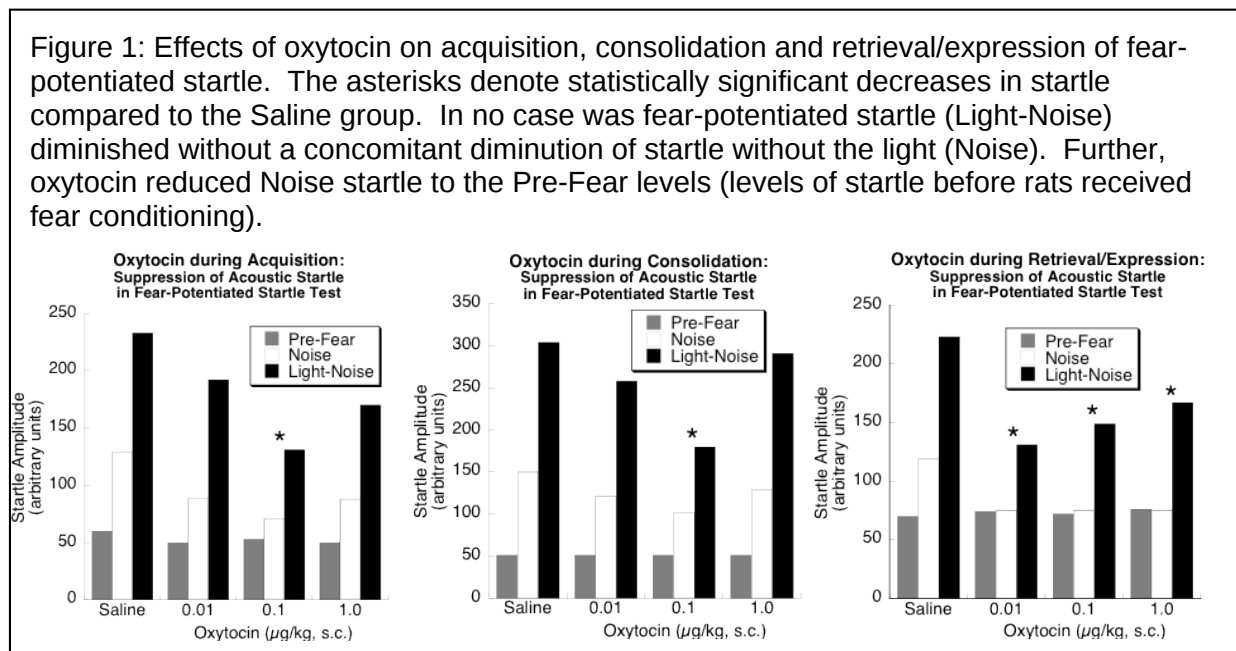
INTRODUCTION

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 anxiolytic 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): Oxytocin Reduces Anxiety-Related Increases in Startle, But Not Cue Specific Fear-Potentiated Startle in Rats (manuscript in preparation).

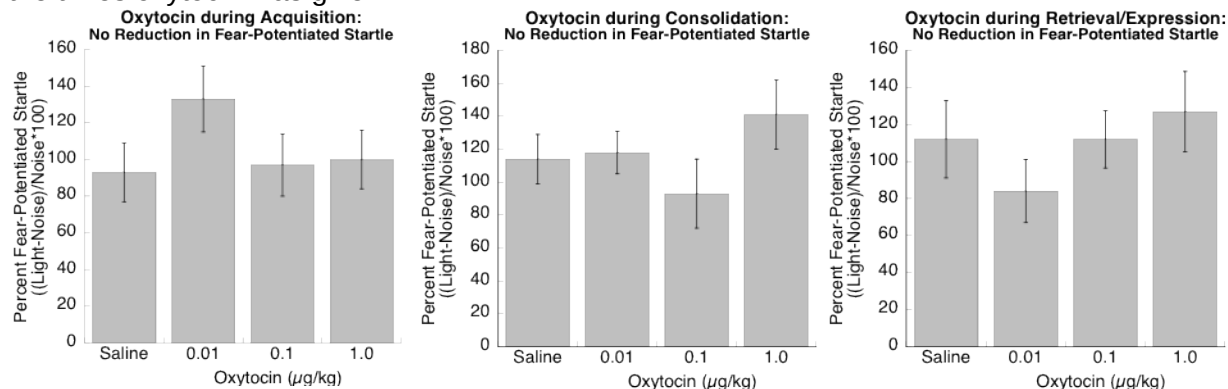
A standard fear-potentiated startle paradigm was used with 12 rats per group in all experiments. In brief, rats were first habituated to acoustic startle stimuli for three days (pre-fear startle). Fear conditioning then occurred with one session of five light-footshock pairings. Four days later, fear-potentiated startle testing occurred where acoustic startle stimuli were delivered in the presence or absence of the light. Greater startle in the presence of the light than in its absence was considered cue-specific conditioned fear. Comparisons of pre-fear startle and startle in the absence of the light (Noise) is a measure of background or generalized anxiety. Greater startle in the absence of the light than during the pre-fear startle sessions is considered increased generalized anxiety. A Dunnett's test was used to statistically compare the doses of oxytocin to that of saline. $P < 0.01$ for all analyses.



To test for the effects of oxytocin on fear conditioning and fear-potentiated startle, oxytocin (0.01, 0.1, 1.0 $\mu\text{g/kg}$, sc) was injected 30 minutes before the light-footshock pairings (acquisition), immediately after the light-footshock pairings (consolidation), or 30 minutes before the fear-potentiated startle test (fear retrieval/expression).

These experiments demonstrated that oxytocin given either during acquisition, consolidation, or expression diminished acoustic startle (Figure 1). The effects were not specific to startle in the presence or absence of the conditioned light stimulus. There was no oxytocin effect on fear-potentiated startle (Figure 2). The results could be interpreted as an effect of oxytocin on the ability to startle or perceive the acoustic stimulus. Another interpretation is that oxytocin diminished background or generalized anxiety induced by the fearful situation.

Figure 2: Oxytocin does not specifically affect fear-potentiated startle when given during acquisition, consolidation or expression of fear-potentiated startle. Percent fear-potentiated startle scores were calculated to normalize the effects of oxytocin on baseline startle in the different groups. There were no statistical differences between any of the groups at any of the times oxytocin was given.



To test these two hypotheses, additional experiments were conducted. In the first experiment, rats were not fear conditioned. Acoustic startle was first tested without oxytocin and then tested again with oxytocin. There was no effect of oxytocin on acoustic startle (Figure 3). This indicates that oxytocin does not diminish the sensory perception or startle response to the acoustic stimulus.

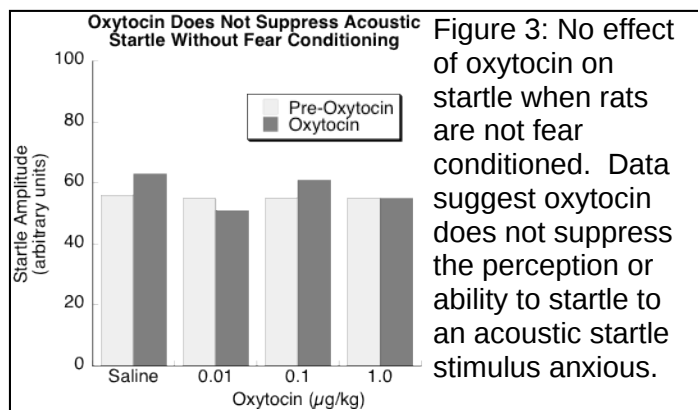
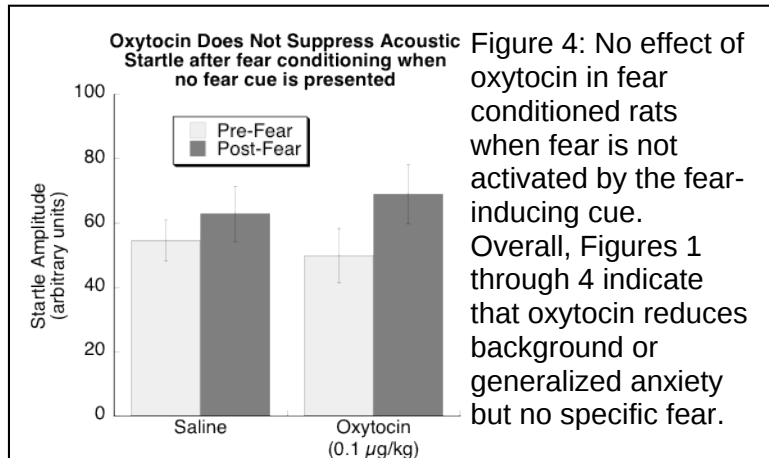


Figure 3: No effect of oxytocin on startle when rats are not fear conditioned. Data suggest oxytocin does not suppress the perception or ability to startle to an acoustic startle stimulus anxious.

In the second experiment, rats were fear conditioned (5 light-footshock pairings), but no light was presented during the acoustic startle testing. There was no effect of oxytocin on acoustic startle when the conditioned fear stimulus was never presented during the testing (Figure 4). Comparing the effect of oxytocin when the situation was made fearful by presenting the light (Figure 1) and when



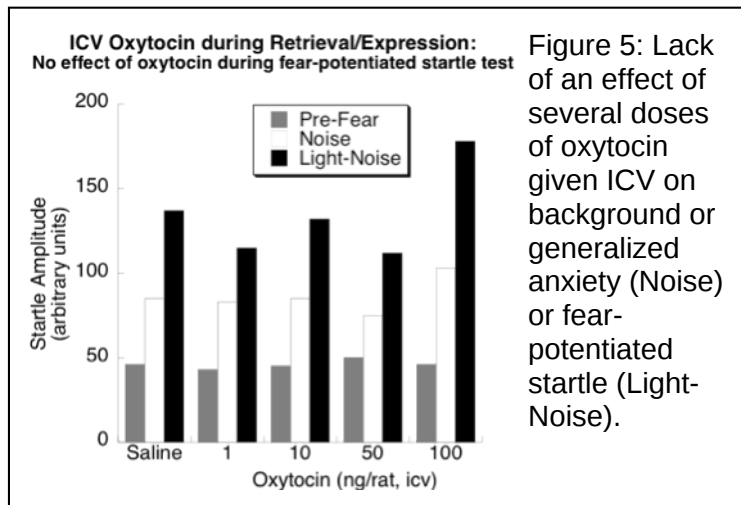
the light was never presented during the startle session (Figure 4), it is concluded that oxytocin diminishes background or generalized anxiety induced by cue-specific fear (Noise and Light-Noise trials in Figures 1 & 2), but does not reduce the explicit fear to the learned stimulus (percent of fear-potentiated startle in Figure 2).

The experiments suggest that oxytocin has therapeutic

implications for PTSD as a unique anti-anxiety drug that relieves generalized or background anxiety, but leaves specific fear unaffected.

Negative results (Task 1): The effects of social support on the effects of oxytocin on fear-potentiated startle were tested in one experiment. Comparison of rats housed in pairs versus isolated rats did not yield differences on the effects of oxytocin on fear-potentiated startle or background anxiety. This experiment needs to be replicated before conclusions can be drawn.

Uncompleted experiments (Task 1): The effects of oxytocin given either intracerebroventricularly (ICV) or into the amygdala (data not show) have begun and are still underway. So, far there is no effect of ICV administration at several doses tested (Figure 5).



However, the highest dose tested, 100 ng, suggests that higher doses might increase startle. We still need to test higher doses. Problems with amygdala injections have been found where the saline injections reduce fear-potentiated startle. We will work on overcoming this problem.

Deviations from the original SOW:

I do not believe we will be able to complete the *in situ*

hybridization or receptor binding experiments originally proposed in Task 2. The additional behavioral pharmacology experiments that were necessary following the results from the fear-potentiated startle experiments, additional ICV and intracranial amygdala infusion experiments necessary to identify appropriate doses of oxytocin, and experiments to solve the amygdala infusion problems will likely reduce the funds available for *in situ* hybridization or

receptor binding experiments. Nevertheless, we will complete corticosterone assays proposed in Task 2. If funds are still available after completion of the behavioral pharmacology studies, as much as possible of the *in situ* hybridization and/or receptor binding experiments will be performed.

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) has presented posters of the research in two University of Delaware forums (the university summer undergraduate research forum and the Department of Psychology undergraduate research forum). The graduate student (Luke Ayers) has presented the studies in the department's seminar series and will be presenting the final version of the studies in a poster at the Society for Neuroscience meeting in Chicago in October. Both will be authors on the published manuscript and participate in all aspects of its production including data collection and analysis, and writing the final article.

KEY RESEARCH ACCOMPLISHMENTS

- Systemically administered oxytocin is an effective antianxiety agent in male rats with unique properties of decreasing generalized anxiety but not cue-specific fear.
- Using a fear-potentiated startle paradigm for conditioned fear, oxytocin was effective in reducing acoustic startle.
- Effectiveness of oxytocin was not confined to one phase of fear learning or expression. It reduced startle when given either before fear conditioning (acquisition, immediately after fear conditioning (consolidation) or during fear expression/retrieval (during fear-potentiated startle testing).
- The reduction in startle was general, in that both startle in the presence of the conditioned fear stimulus and in its absence was diminished. However, cue-specific fear was still evident.
- Further experiments demonstrated that oxytocin did not merely reduce the ability to startle or perceive the acoustic startle stimulus.
- Additional experiments demonstrated that oxytocin only reduced startle in a fear-inducing situation.
- Taken together, it is concluded that oxytocin uniquely inhibits generalized 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 ACCOMPLISHMENTS

- Graduate and undergraduate students (one each) have been trained in behavioral pharmacology using fear-potentiated startle as a paradigm for testing antianxiety drugs.
- They have learned the proper procedures for conducting animal research.
- They have learned how to analyze data, construct posters, and write abstracts and manuscripts.
- The students have learned presentation skills, and will present the research at local and national (Society for Neuroscience meeting) research forums.

REPORTABLE OUTCOMES

1. Rosen, J.B., Missig, G., and L.W. Ayers (2009). Oxytocin reduces anxiety-related increases in startle, but not cue-specific fear-potentiated startle in rats. Presented at the Military Health Research Forum, Kansas City, MO, August 31-September 3. Oral presentation # S3-11 and poster presentation # P19-13.
2. Ayers, L.W., Missig, G., Schulkin, J., and Rosen, J.B. (2009). Oxytocin reduces anxiety-related increases in startle, but not cue-specific fear-potentiated startle in male rats: Relevance to PTSD. Presented at the Society for Neuroscience meeting, Chicago, IL, October 17-21. Program # 841.17.

CONCLUSION

The project has been quite successful. A manuscript is preparation which will present the unique antianxiety effect of oxytocin discovered in our experiments with fear-potentiated startle in male rats. I believe the report will have a substantial impact, as there is tremendous interest in the role of oxytocin in emotions and mental health. Additional experiments are still ongoing to discover a neural locus of action of oxytocin. Participation and training of graduate and undergraduate students has also been successful. Future plans are to leverage the data and results for submissions of a NIH grant proposal and other grant mechanisms through the Department of Defense.

Appendix 1:

Military Health Research Forum Abstract

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

University of Delaware

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.

This work was supported by the U.S. Army Medical Research and Materiel Command under W81XWH-08-1-0182.

Appendix 2:

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.

Supported by: Department of Defense Post-Traumatic Stress Disorder/Traumatic Brain Injury Research Program of the Office of the Congressionally Directed Medical Research Programs grant W81XWH-08-1-0182