Sex differences in fear conditioning in posttraumatic stress disorder

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ABSTRACT

Background: Women are twice as likely as men to develop Posttraumatic Stress Disorder (PTSD). Abnormal acquisition of conditioned fear has been suggested as a mechanism for the development of PTSD. While some studies of healthy humans suggest that women are either no different or express less conditioned fear responses during conditioning relative to men, differences in the acquisition of conditioned fear between men and women diagnosed with PTSD has not been examined.
Methods: Thirty-one participants (18 men; 13 women) with full or subsyndromal PTSD completed a fear conditioning task. Participants were shown computer-generated colored circles that were paired (CS+) or unpaired (CS−) with an aversive electrical stimulus and skin conductance levels were assessed throughout the task.
Results: Repeated measures ANOVA indicated a significant sex by stimulus interaction during acquisition. Women had greater differential conditioned skin conductance responses (CS + trials compared to CS− trials) than did men, suggesting greater acquisition of conditioned fear in women with PTSD.
Conclusions: In contrast to studies of healthy individuals, we found enhanced acquisition of conditioned fear in women with PTSD. Greater fear conditioning in women may either be a pre-existing vulnerability trait or an acquired phenomenon that emerges in a sex-dependent manner after the development of PTSD. Characterizing the underlying mechanisms of these differences is needed to clarify sex-related differences in the pathophysiology of PTSD.

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1. Introduction

Women develop posttraumatic stress disorder (PTSD) at twice the rate of men, despite greater trauma exposure in men (Adamson et al., 2008; Breslau et al., 1998). Fear conditioning has been proposed as a core process in the development of PTSD (Orr et al., 2000; Pitman et al., 2000). During traumatic stress exposure, severe and prolonged threat result in a cascade of biological stress responding (Pitman et al., 2001) leading to the release of stress hormones and neuromodulators that are believed to enhance fear conditioning and memory consolidation, subsequently resulting in chronic symptoms that are characteristic of PTSD (Pitman, 1989;

Pitman et al., 2000). However, little is known about potential sex differences in conditioning mechanisms associated with PTSD.

In fear conditioning, a stressor (i.e., unconditioned stimulus; UCS) elicits fear and arousal (i.e., unconditioned response, UCR) that becomes associated with innocuous cues (i.e., conditioned stimulus; CS) (Orr et al., 2000; Pitman et al., 2000). Subsequently, presentations of the now conditioned stimuli are sufficient to elicit a conditioned response (CR) because of the prior CS–UCS associations. In conditioning models of PTSD, it is believed that neutral environmental cues present at the time of trauma exposure become associated with fear and arousal such that these previously innocuous stimuli can trigger conditioned fear responses even when danger is no longer present (Orr et al., 1997; Vermetten et al., 2007). Individuals who develop PTSD may be more "*conditionable*" in that they are more likely to acquire a conditioned fear response to a neutral stimulus and have more difficulty extinguishing this response once acquired (Orr et al., 2000). While PTSD has been

associated with greater conditioned responses to cues signaling threat in several studies (Orr et al., 2000; Peri et al., 2000), it has not been found in others (Milad et al., 2008; Orr et al., 2006). Some studies have also found that PTSD was associated with greater reactivity to safety signals during fear conditioning, suggesting impaired stimulus discrimination in PTSD (Blechert et al., 2007; Grillon and Morgan, 1999; Peri et al., 2000). However, much of this research has been conducted in males or in studies that failed to examine the impact of sex, which may have led to considerable variability.

Growing evidence suggests that men and women may diverge in their responses to stress and fear acquisition. Brain regions implicated in both fear conditioning and PTSD (e.g., the amygdala, vmPFC, and hippocampus) are sexually dimorphic and contain receptors for sex-specific steroids such as estrogen (Goldstein et al., 2001). Additionally, estradiol, the primary form of estrogen in women during the reproductive years, has been found to modulate fear and extinction learning in animal and human studies (Chang et al., 2009; Milad et al., 2006, 2009; Zeidan et al., 2011). Although not always (Gupta et al., 2001), estradiol has been associated with enhanced trace eyeblink conditioning (Leuner et al., 2004), cued fear conditioning (Jasnow et al., 2006), contextual conditioning (Jasnow et al., 2006), and extinction consolidation (Milad et al., 2009, 2010; Zeidan et al., 2011).

Most studies of sex differences in fear conditioning in humans have examined nonclinical populations. Enhanced acquisition of conditioned fear was found in healthy women compared to men in one study (Guimaraes et al., 1991), while another study found no sex differences (Zorawski et al., 2005). Importantly, these studies did not control for menstrual phase, which is problematic because reproductive hormones can influence conditioning and extinction. A study that controlled for menstrual phase by comparing women in the follicular phase and men found less differential conditioned fear responding during acquisition in women, suggesting that the process(es) underlying or influencing fear conditioning likely differ between men and women (Milad et al., 2006).

The present study examined whether there are sex differences in the acquisition of conditioned fear in men and women with full or subsyndromal PTSD. Women were tested during the early follicular phase to control for the possible confounding effects of changing reproductive hormones in cycling women since estradiol and progesterone are both relatively low in the early follicular phase. Based on women's higher rates of PTSD, we predicted that women with PTSD would have greater acquisition of conditioned fear (larger skin conductance responses to CS+ trials) compared to men with PTSD. Because the model of fear conditioning in PTSD also suggests that greater threat and stress reactivity may affect fear conditioning (Bremner, 2002; Pitman et al., 2001) we also examined whether anticipatory fear of the unconditioned stimulus differs between men and women, and might thereby explain differences in fear conditioning. Because this study was part of a larger clinical trial examining pharmacological agents administered during extinction, we were unable to examine sex differences associated with extinction.

2. Methods and materials

2.1. Participants

Participants were recruited from Veterans Affairs (VA) outpatient and community clinics, and local newspaper and internet advertisements. Potential participants underwent a diagnostic interview, medical history, and laboratory testing for determining study eligibility. Exclusion criteria included organic mental disorder, schizophrenia, bipolar disorder, alcohol dependence, drug abuse or dependence, seizure disorders, neurological disorders, previous moderate or severe head injuries, current infectious illness, and systemic illness affecting CNS function, or any other medical condition known to affect psychophysiological responses. Exclusionary medications included alpha and beta-adrenergic agents, antipsychotics, benzodiazepines, mood stabilizers, anticonvulsants, antihypertensives, sympathomimetics, and steroids. Additional attrition occurred for the following reasons: withdrawal from study (1 male), falling asleep during experiment (3 males), technical difficulties/experimenter error (1 male, 1 female). Data from individuals with very small skin conductance responses to the UCS (i.e., mean response less than .1 μs) (2 females) or to the CS+ (i.e., mean response less than .05 μs) (2 males, 9 females) were also excluded.

We present data from 31 participants (men: $n = 18$; women: $n = 13$), ages 18–65, who met DSM-IV criteria for full or subsyndromal current PTSD as their primary psychiatric complaint and completed the fear conditioning task. Participants reported the following traumatic events that triggered their PTSD symptoms: combat experiences (12), sexual assault (9), physical assault (8), and accidents (2). Comorbid Axis I disorders included: major depressive disorder (6), dysthymia (2), alcohol abuse (1), panic disorder without agoraphobia (1), agoraphobia (1), specific phobia (2), obsessive-compulsive disorder (1), and generalized anxiety disorder (1). Participants were alcohol- and drug-free during testing, as determined from self-report, urine drug screen, and breathalyzer.

2.2. Measures

2.2.1. Psychosocial measures

PTSD symptom levels were determined by interview using the *Clinician Administered PTSD Scale (CAPS)* (Blake et al., 1995). The CAPS provides information on current and lifetime PTSD symptoms and status, providing a diagnosis, frequency, and intensity of symptoms. Full PTSD diagnostic criteria for DSM-IV or subsyndromal PTSD (i.e., CAPS score > 30 and meeting the A1, A2, B, E, and F clusters, and either the C or D clusters) was determined by the CAPS and present for at least 3 months. Psychiatric and substance use disorders were assessed with the *Structured Clinical Interview for DSM-IV, version 2.0 (SCID I-NP)* (First et al., 2002). We used the *Life Stressor Checklist-Revised (LSC-R)* (Wolfe et al., 1996) interview to determine prior exposure to 21 stressful life events (e.g., experiencing or witnessing serious accidents, illnesses, sudden death, physical and sexual assault); whether exposure to events triggered emotions consistent with DSM-IV criterion A2 for PTSD; and the frequency and ages of exposure. All interviews were conducted by Master's-level clinical psychologists, who calibrated their assessments weekly. Self-reported state anxiety prior to conditioning was assessed using the *State-Trait Anxiety Inventory (STAI-state)* questionnaire (Spielberger et al., 1983). The STAI-state includes 20 items assessing feelings of apprehension, tension, nervousness, and worry rated on a 4-point Likert-type scale from 1 (not at all) to 4 (very much).

2.2.2. Psychophysiological measures

A Coulbourn Modular Instrument System was used. Skin conductance was measured directly by a Coulbourn Isolated Skin Conductance coupler (S71-23) using a constant .5 V through 9 mm (sensor diameter) Sensor Medics Ag/AgCl electrodes placed on the hypothenar surface of the participant's non-dominant hand in accordance with published guidelines (Fowles et al., 1981). The SC electrodes were separated by 14 mm. The SC level analog signal was digitized by a Coulbourn Lablinc Analog to Digital Converter (L25-12). A Microsoft Windows-based computer system was used for sampling and storing the digitized SC signal and controlling stimulus presentations.