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Exaggerated Fear-Potentiated Startle Responding in Posttrauamatic Stress Disorder

Author
Luxenberg, Adam A

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Exaggerated Fear-Potentiated Startle Responding in Posttraumatic Stress Disorder

By
Adam Aaron Luxenberg

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Committee in charge:
Professor Ronald Dahl, Chair
Professor Susan L. Ivey
Professor Darlene Francis
Professor Thomas Neylan (University of California, San Francisco)

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Literature Review

Introduction

Posttraumatic stress disorder (PTSD) is a condition that arises in a minority of people following exposure to traumatic events. Exposure to traumatic events is quite common over the course of normal human life. However, only small subsets of those exposed to trauma develop PTSD. PTSD symptoms have long been recognized, and historically, various terms have been used to describe the symptoms. The actual term PTSD, and the clinical definition of the disorder, is a relatively recent development. The symptoms of PTSD include three clusters: hyperarousal; avoidance; and reexperiencing. At present, clinical diagnoses are solely reliant on subjective symptom reports. However, biomarkers have shown some promise in differentiating patients with PTSD.

This literature review aims to discuss the history, epidemiology, and biology of PTSD along with a more detailed discussion about research in the field of PTSD biomarkers. The first section will outline a brief history of PTSD and will review studies regarding the epidemiology of the disorder. That section will be followed by a discussion defining the disorder biologically and clinically. The next element of this review will be a discussion of biomarkers of PTSD and research protocols used to study the disorder. The biomarkers section will begin with a review of findings in acoustic startle studies, discuss fear-potentiated startle as an emerging approach to studying the disorder, and conclude with a review of the fear-potentiated startle literature related to PTSD. The final section of this review will explore gaps in the literature and the next steps necessary to advance research into biomarkers of PTSD. The goal of this review is to provide a detailed account of the current status of PTSD biomarker research that is framed within a broad review of PTSD.

History of PTSD

The symptoms of PTSD have long been recognized over the course of history. The terms "soldier's heart" (Civil War), "shell-shock" (World War I), and "combat fatigue" (World War II) were used in military lexicon before PTSD was recognized as an official psychiatric condition. The official diagnostic criteria for PTSD, based on exposure and outcome, were formally recognized in the *Diagnostic and Statistical Manual of Mental Disorders*-III (DSM) in 1980 (American Psychiatric Association, 1985). The DSM is used to classify mental disorders based on an exhaustive list of diagnostic criteria and is used by many types of health providers in clinical and research settings.

A 2003 review by McNally discusses the emergence of PTSD as a diagnosis and the conceptual basis for it as a DSM syndrome (McNally, 2003). In this review McNally cites studies that chart how military psychiatrists in the era after the Vietnam War began to recognize that the symptoms often attributed to acute stress after war became chronic conditions. A movement to include "post-Vietnam syndrome" in the DSM-III arose out of political pressures and medical findings. The first revision of PTSD diagnostic criteria resulted from the recognition that the disorder was present in people exposed to traumatic experiences outside of military service.

McNally's review pointed out that the diagnostic criteria for PTSD are changing. As it stands, PTSD is one of the only DSM syndromes that specifies a single etiologic event. Since the initial definition of PTSD in DSM-III, the defining characteristics of a traumatic event have been

revised in DSM-IV to include more categories of trauma, and additional connections to trauma. For example, the affected individual does not need to be the person directly involved in the traumatic event, witnessing a traumatic event or being negatively affected by knowledge of a traumatic event qualifies as trauma. The official diagnostic criteria for PTSD are still under revision and will be explored subsequently in this review.

Epidemiology of PTSD

PTSD research seeks to answer why a subset of individuals exposed to trauma go on to develop PTSD. Trauma exposures are events that involve experiencing or witnessing threat to life or physical integrity (American Psychiatric Association, 2000). Trauma is fairly common in the United States, so common that the majority of people in the United States experience an event that can be classified as traumatic over the course of their lifetime. Estimates about prevalence of trauma vary between studies but range from about 50% to about 90% (Breslau et al., 1998; Kessler, Sonnega, Bromet, Hughes, & Nelson, 1995). This section highlights a few of the most prominent epidemiologic studies of PTSD based on populations in North America and in Europe.

Three studies have been particularly important in establishing the prevalence of trauma exposure in the United States. A 1995 study published based on data collected from part II of the National Comorbidity Survey utilized DSM-III criteria for trauma exposure and found that among those surveyed 60.7% of women and 51.2% of men experienced at least one traumatic event over their lifetime (Kessler et al., 1995). A subsequent study in Detroit constructed specifically to investigate trauma and PTSD utilized DSM-IV criteria and found a high prevalence of exposure to at least one traumatic event (89.6% SE 0.8), but great variation in the type of traumatic event experienced (Breslau et al., 1998). A third study in the United States conducted to assess trauma exposure among youth between 2000 and 2002 found an overall 82.5% prevalence of trauma in the population and that unexpected death was the category of traumatic event with the highest percentage (51.9%) of participants reporting (Breslau, Wilcox, Storr, Lucia, & Anthony, 2004). It is clear that trauma exposure is common in the United States. These studies conducted using definitions of trauma from different revisions of the DSM showed slightly different trauma prevalence. However, they indicate that even though the variations are relatively small the important finding is that trauma exposure is high irrespective of the specific way in which it is measured.

Table A: Examples of Traumatic Events

Assaultive Violence
- Rape
- Held captive/tortured/kidnapped
- Shot/stabbed
- Sexual assault other than rape
- Mugged/threatened with weapon
- Badly beaten

Other Injury or Shocking Event
- Serious car accident
- Other serious accident
- Natural disaster
- Life-threating illness
- Child's life-threatening illness
- Witnessed killing/serious injury
- Discovering a dead body

Learning of traumas to close friend/relative
- Close friend/relative raped
- Close friend/relative seriously attacked
- Close friend/relative car accident
- Close friend/relative other accident

Learning about unexpected death

Adapted from: (Breslau, 2004)

Similar studies of trauma exposure have been conducted outside the United States. Some studies have shown similar results, whereas others have shown trauma exposure to be far less common than studies based in the United States. A survey conducted in a midsized Canadian city showed similar rates of trauma to the United States studies, 74.2% of the women and 81.3% of the men reported at least one traumatic event (OR=0.66, 95% CI =0.49–0.89) (Stein, Walker, Hazen, & Forde, 1997). However, a study conducted in 2000 in Germany reported 25.2% (SE 1.3) prevalence of traumatic events in men, and 17.7% (SE 1.1) prevalence of traumatic events in women. Another study, conducted in 1999 in Switzerland, showed that 27.46% (SE 5.03) of males and 28.48% (SE 4.65) of females reported having experienced a traumatic event (Hepp et al., 2006). Both European studies found rates of trauma much lower than found in the United States-based studies. This suggests that trauma, and thus the etiological events that lead to PTSD are influenced by the society in which the individual lives.

PTSD prevalence is much lower than trauma exposure. Two studies have been particularly important in establishing rates of PTSD in samples representative of the United States population. The previously mentioned National Comorbidity Study found the lifetime prevalence of PTSD to be 7.8% (SE 0.5) and the National Epidemiologic survey on Alcohol and Related Conditions showed the prevalence to be 6.4% (SE .18) (Kessler et al., 1995; Pietrzak, Goldstein, Southwick, & Grant, 2011). These findings provide evidence that on a nationwide scale, in the United States, far fewer people develop PTSD than are exposed to traumatic events.

PTSD development following trauma exposures are similar across studies of populations with different trauma rates (Breslau et al., 1998; 2004; Frans, Rimmo, Aberg, & Fredrikson, 2005; Stein et al., 1997). A number of studies have analyzed the conditional probability of developing PTSD given a traumatic exposure. The 1996 Detroit Area study showed that the overall probability of PTSD at any point in time after exposure was 9.2% (SE 1.0) (Breslau et al., 1998). The previously mentioned youth trauma study found the conditional risk of PTSD following any type of trauma to be 10.2% in women and 7.4% in men (OR 1.4, 95% CI 1.0-2.1, P = 0.07) (Breslau et al., 2004). Across multiple studies the conditional probability of developing PTSD with a given traumatic exposure remains at nearly the same level irrespective of population trauma.

The conditional probability of developing PTSD following trauma appears to be much higher for certain traumatic events. The National Comorbidity Survey evaluated rates and impact of trauma exposures by calculating how likely it was that individual trauma categories were the basis for their PTSD assessment. The study showed that certain traumas, such as rape, had very high probabilities of being the basis for PTSD assessment among both men, 62.1% (SE 11.6), and women 74.4% (SE 4.1) (Kessler et al., 1995). Other studies have shown similar results (Pietrzak et al., 2011). The 2000-2002 youth trauma study showed that the conditional probability of PTSD following assaultive violence (15.1%) was much higher than the conditional probability of PTSD following trauma to a loved one (2.9%) (Breslau et al., 2004). These results suggest that the conditional probability for developing PTSD is higher for certain events. Events such as sexual or physical assault are associated with higher risk for developing PTSD than events such as witnessing an event happening to someone else.

PTSD appears to affect women and men at different rates. Studies have shown that the conditional risk of PTSD following trauma is higher in women than in men (Breslau et al., 1998; 2004; Perkonigg, Kessler, Storz, & Wittchen, 2000). The National Comorbidity study showed that women exposed to trauma were more than twice as likely to develop PTSD (20.4% of women, 8.2% of men, z = 6.7, P=.001) (Kessler et al., 1995). The Detroit area study also found that women had roughly twice the conditional rate of PTSD given trauma, and reported that the rate was 13% (SE 1.6) in women and 6.2% (SE 1.2) in men, also suggesting that this difference cannot be described by differential exposure to types of traumas experienced by men versus women, and appears to be due to a greater vulnerability in women to develop PTSD (Breslau et al., 1998). It is not clear what causes this discordance in PTSD rates between men and women.

Although PTSD only affects a minority of people exposed to trauma, it is nonetheless a significant public health problem that affects nearly 8% of the US population. Given the high prevalence of trauma and comparatively low prevalence of PTSD, and our relatively limited understanding of what predisposes people to developing PTSD, it is necessary to find out more about what physiologic differences lead to PTSD in that minority of people who are exposed to trauma.

Clinical Definition of PTSD

The current version of the *Diagnostic and Statistical Manual of Mental Disorders*, DSM-IV, defines PTSD based on six criteria summarized on the following page (American Psychiatric Association, 2000).

Table B: DSM-IV-TR Criteria for PTSD	
Criterion	**Definition**
A	1. Development of characteristic symptoms following exposure to an extreme traumatic stressor involving direct personal experience of an event that involves actual or threatened death or serious injury, or other threat to one's physical integrity; or witnessing an event that involves death, injury, or a threat to the physical integrity of another person; or learning about unexpected or violent death, serious harm, or threat of death or injury experienced by a family member or other close associate. 2. The person's response to the event must involve intense fear, helplessness, or horror (or in children, the response must involve disorganized or agitated behavior)
B	The characteristic symptoms resulting from the exposure to the extreme trauma include persistent reexperiencing of the traumatic event.
C	Persistent avoidance of stimuli associated with the trauma and numbing of general responsiveness.
D	Persistent symptoms of increased arousal.
E	The full symptom picture must be present for more than 1 month.
F	The disturbance must cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.
	(American Psychiatric Association, 2000)

Using an interview based assessment, such as the Clinician Administered PTSD scale (CAPS), patients can be given a definitive diagnosis of PTSD that can be used to make comparisons between groups of those diagnosed with pathologic symptoms (Blake et al., 1995). Although the DSM definition is useful for standardizing the diagnosis between patients and clinics, it is subject to revision on a regular basis, and in the clinical setting, diagnosis is subject to inconsistencies between clinician interviewers. This makes sole use of the DSM criteria difficult for conducting research. An example of this difficulty is the inconsistency between different studies highlighted in the epidemiology section of this review. Since DSM-III and DSM-IV defined PTSD differently, the data have inherent inconsistencies due to the different criteria that were used to classify trauma events.

The current DSM-IV definition is approaching the end of its use, and will be revised for the fifth edition of the DSM (American Psychiatric Association, 2010). Most of the diagnostic criteria will be similar. However, the current draft version of DSM-V includes a comprehensive set of changes affecting some of the specific inclusion criteria. A 2011 review of the proposed changes for PTSD in DSM-V highlights changes under consideration (Friedman, Resick, Bryant, & Brewin, 2011). Two of the more important aspects of the proposed changes are the modification of criterion A1 and the removal of criterion A2. In DSM-IV, Criterion A defines the stressor that leads to PTSD. As is mentioned previously in this literature review, PTSD is one of the few diagnoses in the DSM that identifies an etiological event leading to pathology. In

DSM-IV, Criterion A is divided into objective and subjective components, and was more inclusive than DSM-III with regards to the types of traumatic experiences that could lead to PTSD. In DSM-V, it has been proposed that the objective measure, Criterion A1, be tightened to be less inclusive and that the subjective measure, Criterion A2, be removed. Revisions to the DSM criteria for PTSD are helpful for better characterizing the disorder. However, they also have potentially negative implications for those newly excluded from being included under the diagnosis and for consistency in the realm of research.

Biology of PTSD

PTSD is an anxiety disorder characterized by irregularities in the stress response. A 2011 review of stress and brain plasticity frames stress and its relation to the brain in the following manner (McEwen & Gianaros, 2011). The brain can be thought of as the central mediator of stress resiliency. In this capacity, the brain determines what is threatening, regulates responses to stressors, and makes changes in an attempt to cope with stressful experiences. Inputs from the external environment and internal body systems act on the brain to induce a response that promotes adaptation to the stressor via changes in body systems. Of primary importance to the response mechanism are the hypothalamic-pituitary-adrenal axis (HPA) and the autonomic nervous system. The HPA axis and the autonomic nervous system are thought to be involved in PTSD. This review focuses on a selection of studies that highlight the changes in these systems that regulate stress, and the body structures that control and provide feedback to these stress systems.

The HPA axis has been extensively researched in relation to PTSD because of its role in coordinating neuroendocrine stress response systems. In the normal physiological response to stress, the HPA axis acts first by secreting corticotropin-releasing factor (CRF) from the hypothalamus. CRF then acts on the anterior pituitary to stimulate the production and release of adrenocorticotropin hormone (ACTH), which then stimulates the release of glucocorticoids from the adrenal cortex. The hippocampus, prefrontal cortex, and circulating glucocorticoids (via glucocorticoid receptors) inhibit the HPA axis (Sherin & Nemeroff, 2011). The amygdala and aminergic brainstem neurons stimulate the HPA by increasing CRF in the hypothalamic paraventricular nucleus (Sherin & Nemeroff, 2011).

The normal HPA axis response to stress leads to release of cortisol from the adrenal cortex. It would be expected that if PTSD induced an exaggerated stress response then cortisol would be elevated in PTSD. Paradoxically, cortisol levels are generally found to be lower or at least non-elevated in PTSD in many studies (Yehuda, 2009). Both urinary free cortisol (Mason, Giller, Kosten, Ostroff, & Podd, 1986; Yehuda et al., 1990) and plasma cortisol levels (Boscarino, 1996) have been shown to be low in persons with PTSD.

There are two primary proposed mechanisms for low cortisol findings in PTSD patients and there is increasing evidence that they both are at play. One mechanism is that there is increased negative feedback to cortisol at the hypothalamus and pituitary. Studies of persons with PTSD have indicated that CRF is released at elevated levels, as indicated by increased CRF measured in the cerebrospinal fluid (Baker et al., 1999; Bremner et al., 1997). However, it appears that there may be a blunted ACTH response to released CRF (Smith et al., 1989). The seemingly incongruent finding, that CRF is elevated and ACTH is decreased, can be explained by increased negative feedback response to cortisol. Studies in which dexamethasone, a compound that mimics cortisol's inhibitory effects by binding glucocorticoid receptors, was administered to PTSD patients have shown that there is greater suppression of ACTH and

cortisol in PTSD (Yehuda, Golier, Halligan, Meaney, & Bierer, 2004). Additionally, metyrapone administered to unmask pituitary negative feedback on cortisol has been shown to increase ACTH release in PTSD (Yehuda et al., 1996). These findings suggest that there is a higher level of cortisol negative feedback inhibition of ACTH at the pituitary level via increased glucocorticoid receptor binding in PTSD, in turn this leads to endocrine abnormalities.

Another hypothesis is that the low cortisol levels in PTSD are due to low adrenal output rather than increased pituitary negative feedback. There is mixed evidence for this possibility. One study found PTSD patients with low basal cortisol levels to have no increased sensitivity to glucocorticoid negative feedback as measured with metyrapone testing (Kanter et al., 2001). They suggested that low adrenal release of cortisol accounted for their findings. Another study found both an increased ACTH and cortisol release in response to CRF in PTSD (Rasmusson et al., 2001). A third study showed no differences in the ratio of cortisol/ACTH rebound from acute metyrapone administration in PTSD versus controls suggesting the adrenal function is intact (Neylan, Lenoci, et al., 2003a).

PTSD has also been studied in relation to the sympathetic-adrenal-medullary (SAM) system and the alterations in catecholamines. During exposure to stress, SAM activation results in release of norepinephrine and epinephrine from the adrenal medulla, increased release of norepinephrine from sympathetic nerve endings, and changes in blood flow to many organs (Heim & Nemeroff, 2009). There have been a number of studies demonstrating alterations in baseline catecholamine level or expression in PTSD. Most studies suggest elevated peripheral catecholamine levels in PTSD (Kosten, Mason, Giller, Ostroff, & Harkness, 1987; Yehuda et al., 1998; Yehuda, Southwick, Giller, Ma, & Mason, 1992) However, some studies have suggested that peripheral catecholamine levels are not elevated (Mellman, Kumar, Kulick-Bell, Kumar, & Nolan, 1995; Pitman & Orr, 1990). It is possible that basal catecholamine levels are either normal or mildly elevated. However, studies that have used trauma salient cues in the laboratory have consistently found elevation in PTSD. Another explanation for the discordance in these studies is that peripheral catecholamine levels are not a good measure of physiologic changes in PTSD and that central catecholamine levels are more indicative of changes. One study of cerebrospinal fluid norepinephrine concentration has shown significant elevation in men with PTSD compared to levels found in healthy men (Geracioti et al., 2001).

A 2009 review by Yehuda outlines a possibly unifying theory that could explain the pathophysiology of PTSD in light of the previously discussed biological findings (Yehuda, 2009). In this review, Yehuda proposes that a series of events initiated by low cortisol levels lead to increased sympathetic nervous system activation. Glucocorticoids down regulate catecholamine synthesis and release in the adrenal gland. Thus, decreased cortisol may contribute to exaggerated catecholamine responses. A review by McGaugh and Roozendaal in 2002 explains that a large body of literature suggests catecholamine response may play a role in consolidating memories via activation of the amygdala and hypothalamus (McGaugh & Roozendaal, 2002). Thus, elevated catecholamine levels could then lead a person to a point where memories become "over-consolidated" because of a state of hyperawareness during and following traumatic experiences. This state could essentially become a positive feedback loop where these memories of trauma are continuously remembered and experienced at later times while the sympathetic nervous system is still activated and primed to hyperarousal. This leads to increased fear conditioning to more generalized stimuli seen in PTSD because of the constant hyperawareness and hypervigilance. Yehuda notes that this theory would suggest that cortisol levels and other glucocorticoid alterations in PTSD are pre-trauma characteristics and might explain why only

some of those exposed to trauma develop PTSD. A person with low cortisol and high catecholamine levels before any given traumatic experience could be in a more vulnerable state before trauma that leads them to experience trauma in a way that is more likely to result in PTSD (Yehuda, 2009). It is important to note that this is only one way of interpreting the wide array of previously mentioned findings.

Functional and structural imaging studies have also shown relationships between PTSD and brain changes that could show consequences of some of the previously discussed biological changes. Structural imaging studies of the hippocampus have found that PTSD patients have smaller right hippocampal volume relative to non-trauma non-combat controls, and non-trauma combat controls (Bremner et al., 1995; Gurvits et al., 1996). Interestingly, a study of cortisol levels and hippocampal volume showed that cortisol levels positively correlate with N-acetylaspartate, a marker of hippocampal volume loss, indicating that cortisol may have an effect on hypothalamus volume (Neylan, Schuff, et al., 2003b).

Functional imaging has shown elevated hippocampal activity as measured by regional cerebral blood flow (rCBF) (Shin et al., 2004). One question that has arisen related to hippocampal volume and function has been whether brain structure differences in PTSD patients are caused by the disease or are pre-existing. A recent structural imaging study showed that current PTSD, but not a lifetime history of PTSD, explained differences in the size of the hippocampus (Apfel et al., 2011). This finding is significant because it offers evidence that may be helpful in understanding which conditions are associated with PTSD pathology rather than conditions that predispose an individual to develop PTSD. Other functional imaging studies have focused on the amygdala and medial prefrontal cortex. An fMRI study of these two structures by Shin et al. found evidence to suggest increased amygdala responsivity and diminished medial prefrontal cortex responsivity in PTSD patients compared to controls (Shin et al., 2005).

The body of literature written about PTSD biology lacks clarity. There are many theories about the biology of PTSD. Those theories tend to employ different ways of rationalizing a number of findings, which at times are contradictory. The diversity in theories and findings seems to suggest that there could be multiple factors at play in the development of PTSD. Additionally, dysfunction in different components of the stress regulation systems could mean that there are a number of ways to develop the same pathology. Studies assessing the function, or dysfunction, of each biological components of stress regulation systems have not led to a consensus among those in the field about the biology of PTSD. Research methods that test changes resulting from biological alterations in PTSD, rather than capturing the biological changes themselves, provide another way to approach understanding the mechanisms responsible for PTSD.

Startle

One of the clinical symptoms of PTSD is an exaggerated startle response. Due to the reliability and accuracy with which human startle can be measured, startle was thought to be a logical measure to be used as part of a model in PTSD research. However, studies of acoustic startle in the absence of a contextual manipulation have been unable to show a definitive connection between PTSD and startle reactivity.

The startle paradigm used to research anxiety and fear arises out of the observation that humans seem to startle more to loud sounds when they are afraid; confirmation of this finding was first found in rodent models (Davis, 1986). The acoustic startle reflex is a physiological startle reaction to an unexpected auditory stimulus involving a three-synapse pathway between

the ear, the brainstem, and the facial motor nerves or spinal cord (Chokroverty, Walczak, & Hening, 1992). In order to measure the degree to which the subject is startled, the magnitude of the acoustic startle reflex is measured using physiologic measures. The reflex is measured by the magnitude of response in three measures before and after the stimulus: eye blink electromyogram (EMG), electrodermal conductivity (e.g., skin conductance), and heart rate (Shalev et al., 2000).

Studies have arrived at inconclusive results regarding acoustic startle findings in PTSD. A number of studies have shown greater startle response in PTSD subjects when compared to control subjects (Butler et al., 1990; Metzger et al., 1999; Morgan, Grillon, Southwick, Davis, & Charney, 1996; Orr, Solomon, Peri, Pitman, & Shalev, 1997; Shalev, Orr, Peri, Schreiber, & Pitman, 1992). However, these studies do not all arrive at their conclusions based on the same measures of startle. This inconsistency among results is highlighted in the following studies of startle with regards to EMG measurements. Butler's 1990 study of acoustic startle in PTSD showed exaggerated EMG in PTSD (Butler et al., 1990). However, two other studies showed no difference in EMG measures in PTSD.(Grillon, Morgan, Southwick, Davis, & Charney, 1996; Orr et al., 1997). Similar inconsistencies between studies are evident with respect to skin conductance and heart rate. Shalev's 1992 study of startle showed exaggerated skin conductance and HR in PTSD patients (Shalev et al., 1992), but Orr's 1997 study of startle only shows elevated HR with a slower decline in skin conductance responses over multiple trials but not an exaggerated skin conductance response (Orr et al., 1997).

These inconsistencies suggest that there are aspects of PTSD pathology that are not captured by changes in acoustic startle reactions. Since there is no single outcome variable with consistent significant elevation across all studies, it seems that PTSD likely affects the brain structures involved in the startle response but that there are additional structures involved as well that do not change the acoustic startle response. The inconsistency of results in acoustic startle has led to focus on other potential biomarkers of PTSD including physiologic responses to startle in the context of fear, a fundamentally different way to test PTSD.

Fear-Potentiated Startle Paradigm

The fear-potentiated startle paradigm involves the acoustic startle protocol with the addition of an aversive stimulus to the startle stimulus. In contrast to acoustic startle, fear-potentiated startle tests how the subject contextualizes and assesses threating cues. The addition of a threat elicits fear and results in increased amplitude of the acoustic startle reflex and the recruitment of the amygdala and other medial temporal structures such as the bed nucleus of the stria terminalis (Davis, 1989; Myers & Davis, 2007).The fear-potentiated startle paradigm is particularly useful because the recruitment of additional brain components allows it to capture more potential aspects of pathology in disorders related to fear processing and startle, both of which are potentially critical elements of PTSD pathology. Additionally, the paradigm still can utilize rodent, non-human primate, and human models as was the case in acoustic startle alone (Davis, 1986; Grillon, Ameli, Woods, Merikangas, & Davis, 1991; Winslow, Parr, & Davis, 2002).

The paradigm works by either conditioning a fear response or directly eliciting fear by adding a threat element to the startle testing. In fear-potentiated startle studies the fear response is elicited by pairing an aversive stimulus (i.e., electric shock) with some type of cue (a light, text on a computer screen, etc). After either being conditioned to associate that cue with a shock, or instructed about how the shock is administered, the subject is exposed to the startling tone (or air

puff) in both the presence and absence of the fear condition. Startle is said to be potentiated if the startle response is greater when elicited in the presence of a stimulus paired with threat than when elicited in the absence of the threat (Davis, 1986).

When a single threat condition is used, the study can detect the potentiation of startle in the presence or absence of threat. However, employing a number of threat conditions can be utilized as a way to determine the impact of background threat on startle responses that uncover complexities in the way different situations are perceived (Grillon et al., 1996; Grillon, Morgan, Davis, & Southwick, 1998b; Pole, Neylan, Best, Orr, & Marmar, 2003; Pole et al., 2009). Utilizing a medium threat condition is a way to assess the participant's startle reaction in a situation of ambiguous threat. Data in anxiety disorder subjects such as PTSD have suggested that threat level has the ability to better stratify subjects based on their symptoms (Pole et al., 2003). Using the fear-potentiated startle paradigm under multiple threat conditions reveals information about how the assessment of situational threat based on risk at the time of appraisal is altered in anxiety pathology.

There are a number of benefits to the fear-potentiated paradigm. Since the fear-potentiated startle model can be performed similarly across a number of different species, it is useful as a way to draw connections between experimental studies that could not be conducted in humans (e.g., lesion studies). Results from rodent or primate models can be compared to those with certain symptoms in human and clinical models (Grillon & Davis, 1997). The paradigm is also useful because startle is defined by within-subject changes in trials administered in different contexts. This within-subjects design reduces problems caused by variability between subjects (Davis, 1986). Fear-potentiated startle has been used to study both brain structure and the symptoms associated with disease pathology. Studies have shown that fear-potentiated startle reaction can be altered using a number of drugs including those acting on GABA transmission and adrenoceptors (Davis, Falls, Campeau, & Kim, 1993). Additionally, lesion studies have implicated individual sub-structures within the amygdala as important locations in which conditioned fear is developed and expressed (Davis, 1992).

PTSD and Fear-Potentiated Startle

Fear-potentiated startle is a sensitive measure of startle that incorporates a participant's reaction to contextual threat. Since exaggerated startle is one of the clinical symptoms of PTSD it was thought that the standard acoustic startle paradigm could discern PTSD pathology from normal physiology. However, given inconsistent startle responses in various studies, fear-potentiated startle has been seen as a potentially better way to capture irregular startle in PTSD because of the addition of fear and contextualization (Morgan, Grillon, Southwick, Davis, & Charney, 1995). One hypothesis is that the exaggerated startle in PTSD is best elicited in a fear-potentiated paradigm because of the recruitment of limbic structures involved in the appraisal of fear contexts (Grillon, Morgan, Davis, & Southwick, 1998b). The fear-potentiated startle paradigm has been used as a translational tool to investigate the biology of PTSD as a way to better elucidate differences in PTSD subjects.

Early studies using fear-potentiated startle employed one threat condition. Morgan et al conducted the first PTSD study using fear-potentiated startle with a threat condition and a non-threatening condition. This study found that PTSD subjects reacted with greater EMG response during both baseline and the threat condition than did controls (Morgan et al., 1995). Another study using one threat condition, in which veterans' startle was assessed in a light room and then a dark room, showed that veterans with PTSD and without PTSD had higher startle responses in

the dark than did the civilian control subjects, but not veterans without PTSD (Grillon, Morgan, Davis, & Southwick, 1998a). The findings in each of these studies indicated that additional threat conditions might be necessary to reveal differences between PTSD subjects and those without PTSD. Although these studies suggested that threat conditions were useful in potentiating startle responses, better differentiation between threat conditions was necessary.

Other fear-potentiated startle studies have employed multiple threat conditions. Multiple threat conditions have been used as a way to determine the impact of background threats on fear responses in PTSD. A few protocols have been used to test multiple threat conditions. One method for testing multiple threat conditions in fear-potentiated startle has been employed by Pole et. al. in multiple studies (Pole et al., 2003; 2009; 2007). In this protocol a shock is used as the threat. In the low threat condition the shock electrode is not placed on the subject; in the medium threat condition a shock apparatus is attached to the participant but the subject is presented with a safety cue; and in the high threat condition the shock electrode is attached and the subjects are presented a cue that a shock will be delivered. These studies indicate the following: Current PTSD and current PTSD symptom severity is associated with greater startle responses under low and medium conditions of threat, but not high threat conditions (Pole et al., 2003). When this is taken into consideration, along with previous findings such as elevated startle in both low and high threat conditions (e.g.,(Grillon, Morgan, Davis, & Southwick, 1998a; Morgan et al., 1996)), it appears that differences in startle responses between PTSD and controls are best demonstrated in paradigms that use conditions of ambiguous threat such as the medium condition in this protocol (Pole et al., 2003).

Fear-potentiated startle offers a way to study aspects of PTSD symptoms that may prove crucial to better understanding the biological features of the disorder. By focusing on the changes that occur in PTSD due to alterations in biological systems, this paradigm offers a way to study the disorder in a way that avoids some of the problems that have arisen in studying the biology of PTSD. Particularly intriguing are the findings that suggest exaggerated startle in ambiguous threat conditions could represent an inability to ascertain the safety of a given situation in PTSD. Additional research that better characterizes how this inability is related to development and maintenance of the disorder is necessary.

Areas for Additional Research

Exaggerated fear-potentiated startle under ambiguous threat and failure to habituate to the acoustic stimulus may be vulnerability factors for PTSD. Pre-trauma exaggerated fear-potentiated startle responses and slower habituation to the acoustic stimulus predicted greater PTSD symptom severity following trauma (Pole et al., 2009). In order to examine which aspects of fear-potentiated startle are associated with current PTSD as opposed to vulnerability to PTSD, it is would be helpful to compare samples with current PTSD and samples with PTSD in remission.

Conclusion

This literature review has outlined the current status of the fear-potentiated startle paradigm in PTSD research and has provided background for selected aspects of PTSD. The defining characteristics of PTSD are being continuously revised as research focused on better characterizing the disorder finds new attributes of the pathology. Biomarkers hold promise as a way to better characterize PTSD and detect the changes in physiology that might predispose an individual to developing the disorder. A central question in PTSD remains to be answered. Why

do a significant minority of those exposed to trauma go on to develop the disorder? This literature review has explored some of the aspects of the disorder that are well characterized in the literature and has suggested areas that should be pursued in order to contribute to answering that central question in PTSD research.

The next step will be to study fear-potentiated startle in participants with current PTSD, remitted PTSD, and no history of PTSD. This study will explore the hypothesis that exaggerated fear-potentiated startle is a feature of current PTSD that is absent in those without current PTSD. By including a remitted PTSD group the findings will be able to suggest whether or not exaggerated startle normalizes with recovery. The findings in this study will have the potential to enhance our understanding of the biology and clinical diagnosis of PTSD.

Original Research

Introduction

PTSD Background and Prevalence

Posttraumatic stress disorder (PTSD) is a condition that arises in a minority of people following exposure to traumatic events. Although a majority of people experience at least one traumatic event in their lives, the lifetime prevalence of PTSD in the United States is estimated to be around 5%-10% (Breslau et al., 1998; Kessler et al., 1995; Kessler, Chiu, Demler, & Walters, 2005). Differences between individuals who develop versus do not develop PTSD, and between individuals with current versus remitted PTSD remain poorly characterized. The present study employs a fear-potentiated startle protocol to assess startle responses to an acoustic stimulus in participants with no history of PTSD, remitted PTSD, and current PTSD.

Startle

PTSD symptoms in the DSM-IV are categorized into three clusters: hyperarousal, avoidance, and re-experiencing of the precipitating traumatic event (American Psychiatric Association, 2000). Hyperarousal is one of the clinical symptoms of PTSD that has been utilized to study PTSD in research settings through exaggerated startle responses to acoustic stimuli. The acoustic startle reflex is a physiological reaction to an unexpected auditory stimulus involving a three synapse pathway between the ear, the brainstem, and the facial motor nerves or spinal cord (Chokroverty et al., 1992; Davis, Gendelman, Tischler, & Gendelman, 1982; Hoffman, Cohen, & Stitt, 1981). The reflex is measured by the magnitude of response in three measures before and after stimulus: heart rate, eye blink electromyogram (*orbicularis oculi*), electrodermal conductivity (e.g. skin conductance) (Shalev et al., 1992). Many studies have shown elevated startle response in PTSD subjects compared to controls (Butler et al., 1990; Metzger et al., 1999; Morgan et al., 1996; Orr et al., 2003; Shalev et al., 1992) However, not all studies have demonstrated elevated startle responses in PTSD across all measures of the response (Grillon et al., 1996; Orr et al., 1997). The basic startle reflex involves a limited number of brain structures and it has been theorized that fear enhanced startle comes from the recruitment of related brain structures, rather than those directly responsible for the reflex (Davis, 1989).

Fear-potentiated startle is a research protocol that adds threat conditions to the acoustic startle protocol in order to examine the regulation of the startle reflex. This differs from acoustic startle because the fear-potentiated startle protocol tests how the subject contextualizes and assesses threatening cues, tasks possibly altered in PTSD states. The addition of threat elicits fear, and results in increased amplitude of the acoustic startle response, as well as recruitment of the amygdala and other medial temporal structures such as the bed nucleus of the stria terminalis (Davis, 1989; Myers & Davis, 2007). Exaggerated fear-potentiated startle has been shown in participants with PTSD with noncontextual and context-specific threat conditions (Grillon & Morgan, 1999; Grillon, Morgan, Davis, & Southwick, 1998b; Morgan et al., 1995). Related studies have further classified the finding of elevated startle in protocols that include fear conditions by employing a number of threatening conditions of different intensity showing that elevated startle may only be present in certain types of threat conditions, particularly those in which ambiguous threat is involved (Pole et al., 2003; 2009).

Exaggerated fear-potentiated startle under ambiguous threat may not only be present in PTSD, but may represent stable vulnerability factors for PTSD present in those predisposed to developing PTSD. Pre-trauma startle reactivity as measured by both skin conductance reactivity

and habituation has been shown to be predictive of PTSD symptoms following trauma exposure in a study utilizing fear-potentiated startle (Pole et al., 2009). In order to examine which aspects of fear-potentiated startle are associated with current PTSD as opposed to recovery from PTSD, it is necessary to further examine this issue.

The present study aims to compare fear-potentiated startle reactivity in participants with current PTSD versus PTSD in remission across multiple threat conditions and to study the relationship between symptoms and startle reactivity. We hypothesize that exaggerated startle response to ambiguous threat is an indicator of PTSD vulnerability and expect there to be elevated startle responses in both current and remitted PTSD participants. Specifically, we predict that participants with remitted PTSD will exhibit startle responses similar to those with current PTSD in conditions of low and ambiguous threat, and that both groups will exhibit significantly higher startle responses under low and ambiguous threat compared to participants with no PTSD. We expect no significant differences in responding between groups in the high threat condition as this is the expected response in a dangerous situation. Secondarily, we predict that there will be a significant correlation between severity of PTSD symptoms and startle responses in patients with current and remitted PTSD, and that patients with a higher lifetime trauma exposure will have higher startle responses.

Methods

Study Design

Our study utilized data from a cross-sectional study of male and female First Persian Gulf War veterans aged 31 to 71 collected between 2002 and 2007 (n = 396). We analyzed data from a subset of study participants within three groups: current PTSD (n=48); remitted PTSD (n=42); and no PTSD with no history of exposure to a traumatic event involving threat to life or physical integrity that elicited fear, helplessness, or horror (n = 97).

The study from which our data is sourced was an investigation of the impact of Gulf War Illness on the brain (Weiner et al., 2011). More information about specific features of the protocol that are not directly relevant to our use of the data can be found in the original study (Weiner et al., 2011). The Veterans Affairs Medical Center and the University of California, San Francisco Committees on Human Research, and the Department of Defense Human Subjects Research Review Board approved the protocol.

Participants

Participants were recruited via convenience sampling though contacts with physicians at Veterans Affairs clinics and hospitals, and from a list of veterans furnished by the Department of Defense. The sole inclusion criterion was status as a US Veteran of the First Persian Gulf War. The exclusion criteria were: severe physical impairment, medical illnesses thought to be responsible for symptoms of Gulf War Illness, current or lifetime history of a psychiatric disorder with psychotic features, presence of suicidal or homicidal ideation, use of anti-psychotic medications during the past 6 weeks, history of neurologic or systemic illness affecting CNS function, history of head injury associated with prolonged loss of consciousness, issues with participating in MR imaging studies, current substance other than alcohol abuse, or being a pregnant or nursing mother.

Clinical Tests

The Clinician Administered PTSD Scale (CAPS) was used to ascertain both current and lifetime PTSD symptoms as defined by DSM-IV (Blake et al., 1995). The score can be used as both a continuous measure of symptoms and a categorical measure of PTSD status.

Trauma History

Trauma history was ascertained by self-report using the Trauma History Questionnaire (THQ) (Green, 1996). The THQ asks the participant to respond to 24 questions that ask about specific types of potentially traumatic experiences. The participant marks whether or not they have experienced a given type of trauma, and if it they have, the age at which the type of trauma began and ceased, and how many events of that type they experienced.

Startle

All participants included in this analysis participated in a startle protocol administered by research technicians. In preparation for the startle protocol participants were asked to refrain from activities and substances that might impact results. These activities included exercise, cigarettes, and coffee on the day of testing, and eating within 1 hour of testing. Their compliance was assessed by self-report.

The startle apparatus consisted of a visual threat cue and devices to deliver audio and shock. The visual threat cue was displayed on a computer monitor placed a few feet away from the participant. Participants were asked to fixate their attention on the monitor during testing. Headphones covering both ears were used to deliver auditory stimuli of 40-ms white noise bursts at 115-dB(A) with 0-ms rise and fall times administered to both ears over 70-dB(A) background noise. All tones were generated by a San Diego Instruments Startle Reflex System (SR-Lab). A Coulbourn Instruments Transcutaneous Aversive Finger Stimulator Model E13-22 worn on the second and third digits of the dominant hand was used to administer 2.5 mA shocks following the final acoustic stimulus in the high threat condition.

Measures

Startle was measured using three outcome variables: heart rate (HR), eye blink electromyogram (EMG), and skin conductance (SC).

Heart Rate - HR is used as a measure of sympathetic nervous system activity. HR in beats per minute was obtained by 3-Dot model, 3M Corporation (Maplewood, MN) electrocardiogram electrodes attached in a type I EKG configuration. The signal was filtered for 8 Hz to 13 Hz activity and amplified by 10,000 by a Coulbourn Instruments V75-04 Isolated High Gain Bioamplifier. The interbeat intervals were calculated by software from R-peaks of the digital EKG signal and converted to HR measurements.

Electromyogram - EMG is a measurement of the voltage associated with muscle contractions of the two *orbicularis oculi* muscles. It was measured using three 4-mm sensor diameter InVivo Metrics Ag/AgCl surface electrodes that were filled with Signa Gel electrolyte paste. Two of the electrodes were placed on the left *orbicularis oculi* muscles and a ground electrode was placed behind the left ear. The signal was amplified by 10,000, rectified, and filtered to keep the range between 10 and 500-Hz. Impedance levels were kept below 10kΩ and notch filtered at 60Hz by a Coulbourn Instruments V75-04 Isolated Bioamplifier. The signal was digitized for 4s beginning

at stimulus onset, smoothed by a 5-ms time constant added by a Coulbourn Instruments V76-23A Contour Following Integrator, and stored for off-line analysis.

Skin Conductance - SC is an index of electricity conduction through skin over time. The measure varies with changes in sweat gland activity. An Expanded Technologies SC coupler delivering 0.5 V through 9-mm sensor diameter InVivo Metrics Ag/AgCl electrodes filled with Signa Creme was attached to the medial phalanges of the left middle and index fingers. SC voltages were sampled at 500 Hz and amplified by a VI-MasterLab. The results were stored for off-line analysis.

Sampling

Each of the three measures was sampled at 2 Hz during the resting period and at 1000 Hz during the stimulus presentation. Human Startle Software by Coulbourn Instruments generated means values for each measure in the 1s prior to stimulus onset and mean values in the 1 to 4 s post stimulus for SC and HR, and 21ms to 200ms for EMG.

Conditions

All subjects were assessed with the startle protocol under low, ambiguous, and high threat of shock. At the beginning of the protocol participants were told that they would receive shocks later in the study but that they could not occur until they were fitted with the shock device. For the low threat condition participants went though the startle procedure with no finger shock device and were specifically told that they would not be shocked. In the ambiguous threat condition participants were fitted with the finger shock device but were signaled via text on the monitor that indicated they would not be shocked (i.e., "NO SHOCK"). Under the high shock condition the participants were fitted with the finger shock device and signaled with a message that indicated that shocks were possible (i.e., "SHOCK POSSIBLE"). Before beginning this phase, participants were told that when shocks were possible it would entail up to three shocks at any point in time. Shock was delivered following the auditory cue in the high threat condition. The monitor remained on one of the shock indicator messages for the duration of the ambiguous and high threat conditions. All participants underwent the low threat condition first for ten trials, followed by the ambiguous or high threat condition for five trials each. Ambiguous and high conditions were administered in a counterbalanced manner.

Data Reduction

All startle response values (HR, EMG, and SC) were square root transformed to reduce skew created by large response values. Outlier values, as defined by responses outside of three standard deviations to the mean, were removed for EMG and SC data. These outlier responses were assumed to be due to recording error in the measurement device.

Statistical Approach

Our analysis employed analysis of covariance (ANCOVA) and partial correlations. In order to compare group differences in startle and to compare within-subjects differences across startle conditions we used one-way ANCOVA covarying for the sociodemographic variables that differed between groups. To analyze correlation between startle magnitude and PTSD symptoms, and to analyze correlation between startle magnitude and reported trauma categories, we used

partial correlations also covarying for sociodemographic variables that differed between groups. The tests are described in more detail below.

ANCOVA Between PTSD Groups within Threat Level

Using a one-way analysis of covariance (ANCOVA) we examined group differences in mean startle response with respect to HR, EMG, and SC. In order to control for potential confounds that differed significantly between PTSD groups and correlated with at least one outcome variable, we assigned these variables as covariates. Covariance analysis was also conducted on variables for which there were data missing. Separate analyses were performed at each threat level to determine whether or not there were group differences based on PTSD at a given threat level. Planned comparisons were performed to analyze the difference between the current PTSD group and the two other groups, no PTSD and remitted PTSD.

Correlation between Startle Response and PTSD Symptoms

We assessed correlations between startle response and PTSD symptoms for participants with current PTSD and remitted PTSD in each of the threat levels. We used partial correlations that covaried for potential confounds, and performed separate analyses for each of the three threat conditions (low, ambiguous, and high) under each of the measures of startle (EMG, SC, HR).

Correlation between Startle Response and Number of Categories of Trauma

In order to assess the relationship between the startle response and lifetime trauma exposure, we used partial correlations. Correlations were performed for each threat level (low, ambiguous, and high) under each measure of startle (HR, EMG, and SC).

All analyses were conducted with IBM SPSS Statistics version 20.0 (Armonk, New York) and the significance level was set at $p < .05$.

Results

Descriptive Statistics

The descriptive statistics tables present mean values for the variables that describe the sample population. Table 1 presents values that describe the sample population without grouping by PTSD status. Table 2 presents values that are grouped by PTSD status and analyzed using analysis of variance to allow us to determine descriptive variables with values that differ between PTSD groups in our sample.

Table 1: Descriptive Statistics Across all Participants				
	N	Mean (SD) or %	Minimum	Maximum
Age	187	44.66 (9.63)	31	71
Years of Education	180	14.59 (2.42)	11	34
Missing	7			
Gender				
Male	162	86.6		
Female	25	13.4		
Ethnicity				
Caucasian	109	58.3		
African American	32	17.1		
Latino	18	9.6		
Other	19	10.1		
Missing	9	4.8		
Childhood Trauma	38	20.3		
Lifetime CAPS	187	34.6 (39.6)	0	125
Current CAPS	187	20.2 (27.4)	0	108

Table 2: Descriptive Statistics by PTSD Group				
	No PTSD	Remitted PTSD	Current PTSD	P-Value of ANOVA
Number of Participants	97 (51.9%)	42 (22.5%)	48 (25.7%)	
Age	46.3 (9.62)	44.9 (10.23)	41.2 (8.30)	.01
Female Gender	12 (12.4%)	5 (11.9%)	8 (16.7%)	ns
Years of Education	15.1 (2.88)	14.0 (1.51)	14.0 (1.82)	.02

Potential Confounds

Mean values for age and years of education were significantly different between PTSD groups. Due to these differences both variables are potential confounds in our analysis of startle

differences. An analysis of the correlation between age and startle response, and separately years of education and startle response, showed that for both age and years of education there was a significant correlation with startle response in at least one of the measures. Due to the significant correlation both age and years of education were modeled as covariates in all analyses.

Difference in Startle Response between PTSD Between Threat Level

Table 3: Means at Each Threat Level Across all Measures and Groups

	HR		*EMG*		*SC*	
	M	*SD*	*M*	*SD*	*M*	*SD*
Low						
No PTSD	0.13	0.22	1.04	1.34	0.03	0.03
Remitted	0.15	0.20	1.23	1.40	0.04	0.04
Current PTSD	0.23	0.21	1.29	1.84	0.04	0.04
Ambiguous						
No PTSD	0.15	0.15	1.44	1.82	0.03	0.03
Remitted	0.16	0.17	1.65	1.88	0.04	0.03
Current PTSD	0.28	0.28	1.61	1.93	0.05	0.05
High						
No PTSD	0.20	0.26	1.62	2.11	0.04	0.04
Remitted	0.19	0.22	2.08	2.19	0.05	0.05
Current PTSD	0.23	0.23	1.66	1.96	0.05	0.04

HR

Table 4: ANCOVA Table – HR at Each Threat Level with Planned Comparison Groups

	Df	*F*	*t*	*p*
Low	2	2.90		0.06
Current vs Remitted	171		-2.33	0.02*
Current vs No PTSD	171		-1.82	0.07
Ambiguous	2	4.73		0.01*
Current vs Remitted	171		-2.93	< .01*
Current vs No PTSD	171		-2.42	0.02*
High	2	0.120		0.89
Current vs Remitted	171		-0.12	0.91
Current vs No PTSD	171		-0.46	0.65
* indicates significance at p=.05 level				

Under the low threat condition there was no significant overall group difference in HR startle responses, but there was a trend towards a group difference $F(2,171)= 2.90$, $p= .06$. In planned comparisons, startle response in the current PTSD group was significantly higher than the no PTSD group, $t(171)=-2.33$, $p= .02$, and trended towards being higher in the remitted PTSD group, $t(171)=-1.82$, $p= .07$.

In the ambiguous threat condition there was a significant overall group difference, $F(2,171)=4.73$, $p= .01$. The current PTSD group demonstrated significantly higher startle

response than both the remitted PTSD group t(171)=-2.42, p= .02 and the no PTSD group t(171)=-2.93, p< .01.

There were no significant differences between PTSD groups in the high threat condition with respect to HR measurements.

EMG

Table 5: ANCOVA Table – EMG at Each Threat Level with Planned Comparison Groups				
	Df	*F*	*t*	*p*
Low	2	0.26		0.77
Current vs Remitted	114		-0.61	0.55
Current vs No PTSD	114		-0.005	> .99
Ambiguous	2	0.161		0.85
Current vs Remitted	114		-0.32	0.75
Current vs No PTSD	114		0.21	0.83
High	2	0.65		0.52
Current vs Remitted	114		-0.05	0.96
Current vs No PTSD	114		0.92	0.36

There were no significant group differences between PTSD groups in any of the threat conditions with respect to EMG.

SC

Table 6: ANCOVA Table – SC at Each Threat Level with Planned Comparison Groups				
	Df	*F*	*t*	*p*
Low	2	1.58		0.21
Current vs Remitted	171		-1.61	0.12
Current vs No PTSD	171		-0.29	0.77
Ambiguous	2	1.58		0.21
Current vs Remitted	171		-1.78	0.08
Current vs No PTSD	171		-1.1	0.27
High	2	1.48		0.23
Current vs Remitted	171		-0.94	0.35
Current vs No PTSD	171		0.68	0.5

There were no significant group differences between PTSD groups in any of the threat conditions with respect to SC.

Correlation between Current PTSD Symptoms and Heart Rate Startle Response

In analyses including only participants with current or past PTSD who had current CAPS across the entire range from 0 to 108 (M = 41.0, SD 25.4), we found there was no significant correlation between severity of current PTSD symptoms and higher HR startle responses in any of the threat conditions when adjusting for age and education. There was a non-significant correlation at trend level in the ambiguous threat condition in HR startle, r = 0.20, p = .07. There were no significant correlations at any threat level in either the SC or EMG measures.

Correlation between Number of Trauma Categories and Heart Rate Startle Response

The partial correlation between the number of trauma categories reported was significant in the ambiguous threat condition of HR startle measurement $r=.23$, $p< .01$. The HR startle measurement did not significantly correlate with number of trauma categories in the low or high threat condition. There was no significant correlation between current PTSD symptom severity and startle as measured by SC or EMG.

Discussion

The present study indicates that exaggerated autonomic startle under ambiguous threat varies as a function of the presence of current PTSD. Also under the ambiguous threat condition, the magnitude of the autonomic startle response correlates with the number of reported trauma categories. Our findings were not supportive of the theory that exaggerated fear-potentiated startle is a vulnerability factor for developing PTSD. Our investigation was novel in a number of ways. The fear-potentiated startle protocol has often been used to study PTSD and most frequently to characterize participants with current PTSD versus participants without PTSD (Grillon, Morgan, Davis, & Southwick, 1998a; Morgan et al., 1995). This study applied this protocol to a population in which there were participants with current PTSD, remitted PTSD, and no PTSD. This gave our study the ability to assess whether differences between current PTSD responses and remitted PTSD responses are supportive of fear-potentiated startle responding as a vulnerability factor for PTSD. Our study also was novel in its evaluation of the correlation between the magnitude of startle response under ambiguous threat and lifetime trauma exposure.

The present study builds upon a large body of evidence indicating exaggerated fear-potentiated startle in PTSD. Our data provide further evidence that startle is elevated in people with current PTSD versus people without PTSD, as has been shown in previous investigations (Grillon, Morgan, Davis, & Southwick, 1998a; Morgan et al., 1995). Our findings also indicate that under ambiguous threat HR startle response allows for differentiation between the current PTSD and remitted PTSD groups, in addition to differentiation between the current PTSD and no PTSD groups. A previous study of acoustic startle in monozygotic twins in which one twin had combat exposure and PTSD and one twin had no combat exposure and no PTSD found exaggerated HR startle only in the twin with PTSD, supporting the hypothesis that exaggerated HR startle could be an indicator of current PTSD rather than genetic vulnerability for PTSD (Orr et al., 2003). Our findings reach a similar conclusion using the fear-potentiated startle procedure. The present study also shows that HR response in the ambiguous threat condition correlates with number of reported categories of lifetime trauma exposures. This could be due to higher exposure to trauma in participants with current PTSD. Additional studies investigating relationships between trauma, PTSD, and fear-potentiated startle are necessary to better characterize this relationship. These findings provide evidence that differences in HR startle under ambiguous threat could be a useful indicator of current PTSD pathology.

Our study did not show similar results in either EMG or SC. There were no significant group differences between startle responses between current PTSD and no PTSD using the SC and EMG measures of startle in our study as has been shown in previous investigations (Grillon, Morgan, Davis, & Southwick, 1998a; Morgan et al., 1995; Pole et al., 2003). It has been proposed that SC should not be used to measure startle in fear-potentiated startle procedures primarily due to the possibility that SC is not as sensitive to variations in amygdala regulation of the startle reflex (Glover et al., 2011). Our data also indicate that SC is not useful in differentiating startle response related to trauma and PTSD. HR may be a better measure of

autonomic startle when using fear-potentiated startle procedures. It is possible that HR is a better tool to measure amygdala regulation of the startle response.

Our study expands findings in previous studies that investigate the utility of multiple threat conditions, and particularly ambiguous threat conditions in evaluating PTSD. Previous studies have reported that ambiguous threat could be used to distinguish those vulnerable to PTSD from those not vulnerable before exposure to trauma (Pole et al., 2009). Our data suggest that multiple threat conditions have utility in the fear-potentiated startle protocol. In our analysis of mean differences between HR startle among PTSD groups we saw significant group differences in the ambiguous threat condition that were not present in either the high or low threat conditions. This finding highlights the utility of an ambiguous threat condition as it may be a way to test PTSD pathology related to fear processing and assessment. The advantage of using ambiguous threat conditions may stem from characteristics of PTSD pathology. The ambiguous threat condition requires participants to trust that the signal indicating no shock is possible is trustworthy and represents reality. PTSD may make it more difficult, or impossible, to trust the safety signal. This perhaps explains why participants with PTSD responded to the startling tone in the ambiguous threat condition in a manner that more closely resembled responses to the high threat condition in other groups, thus there was an inability to encode the safety signal due to some dysfunction in the higher order fear processing centers in the brain.

Our study found a correlation at trend level between HR startle response and current PTSD symptom severity in participants with either current PTSD or remitted PTSD in the ambiguous threat condition. Additional symptoms in PTSD have been shown to be associated with negative life events and poor quality of life (Lunney & Schnurr, 2007; Schnurr, Hayes, Lunney, McFall, & Uddo, 2006). Since PTSD diagnosis relies upon the presence or absence of a specific number of symptoms in certain categories present for defined durations, it is possible that the presence or absence of a single symptom could exclude or include a person in a PTSD diagnosis. A measure that correlates with symptoms could be useful in tracking symptom improvements with treatment even if the diagnosis category does not change. The finding of trend level association between HR startle and severity of current PTSD symptoms provides further evidence that the protocol could be used to gauge the severity of current PTSD pathology. However, the non-significant finding must be interpreted as only an indicator that more research with respect to the relationship between HR startle and current PTSD symptomology is necessary.

It is hypothesized that PTSD pathology impacts the brain structures involved in fear and emotion regulation as well as in the startle reflex, including the amygdala and hippocampus among other structures (Heim & Nemeroff, 2009). However, the extent to which dysfunction in the regulation or the function of these structures predisposes one to PTSD, rather than being an impairment acquired along with PTSD, is debated. The present study provides evidence that exaggerated fear-potentiated HR startle responses vary as a function of current PTSD. This does not support the hypothesis that exaggerated startle demonstrated in ambiguous threat conditions is a marker of PTSD vulnerability. This fits with findings from a recent study showing that current but not remitted PTSD was associated with smaller hippocampus volume (Apfel et al., 2011). Further studies, particularly longitudinal studies following participants before PTSD, are necessary to provide additional support for the vulnerability hypothesis.

Any discussion of measurements that can be used to differentiate people with PTSD from those without PTSD raises the question of the utility of the test for diagnostic purposes as a biomarker. Currently, clinical diagnosis of PTSD relies upon subjective symptom reports and

interview by an extensively trained provider. A biomarker of PTSD could be used in clinical settings to provide screening for those with potential PTSD or to identify those at risk for developing PTSD. Some recent studies have described potential biomarkers including one related to fear-inhibition impairment in startle (Jovanovic, Kazama, Bachevalier, & Davis, 2012; van Zuiden et al., 2011). The results of our study suggest that the HR measure in a fear-potentiated startle test could show differences between patients with and without PTSD under conditions of ambiguous threat. The measure potentially could be used as a component of a larger biomarker testing protocol. HR is particularly intriguing because it is easily measured using tools presently available in many clinical settings such as EKG machines or even HR monitors sold for use as a fitness monitor. Further research is required to better characterize the utility of fear-potentiated startle measurements as a viable component of a biomarker test and the potential use of a number HR measurement devices.

There are limitations to the conclusions that can be drawn from the presented data. One such limitation is the fear-potentiated startle protocol itself. As discussed previously, prior studies of fear-potentiated startle in PTSD have not shown elevations in a single measurement or in a certain threat condition. Since our study did not demonstrate elevated SC and EMG responses in PTSD, our results have to be interpreted with this caveat.

Our study design also had limitations. The classification of PTSD status was subject to limitations related to recall bias and misclassification due to the limits of a single interaction between interviewers and participants. PTSD status is better established over multiple interactions between provider and patient. The trauma history questionnaire that was used to ascertain trauma history in our population is also subject to recall bias and because it is self-reported it is subject to inconsistency between respondents. Additionally, we looked for evidence supportive of exaggerated fear-potentiated startle as a vulnerability factor for developing PTSD by comparing current PTSD participants to remitted PTSD participants. It is possible that the process of recovering from PTSD induces changes that mask any differences that potentially exist in PTSD susceptible and PTSD vulnerable populations. This study also compared groups of participants with PTSD to a group of participants without PTSD and without trauma exposure. Limited conclusions can be drawn about these comparisons since those with trauma exposure in the remitted PTSD and current PTSD were compared to a control group without trauma exposure. Another potential limitation of our study is the population from which participants were sampled. As all participants were veterans of the First Gulf War, it is expected that trauma exposure is more prevalent than in a non-veteran population. Furthermore our sample was comprised primarily of men and hence generalizability to female populations is limited. It is also possible that our exclusion criteria introduced some bias into our sample population as participants with hyperarousal may be more likely to take substances to control their symptoms.

Conclusion

This study indicates that exaggerated autonomic startle response under ambiguous threat varies as function of current PTSD and the response correlates with the number of past trauma categories experienced. Our study does not support the theory that exaggerated startle response is a marker of vulnerability to PTSD. The finding that heightened autonomic startle response is characteristic of all groups under high threat, does not distinguish between all groups under low threat, and is only present in the current PTSD group under ambiguous threat suggests that PTSD is characterized by impaired ability to accurately judge safety cues. This study suggests that

measuring autonomic startle responses under ambiguous threat has potential utility for gauging PTSD status in a clinical setting.

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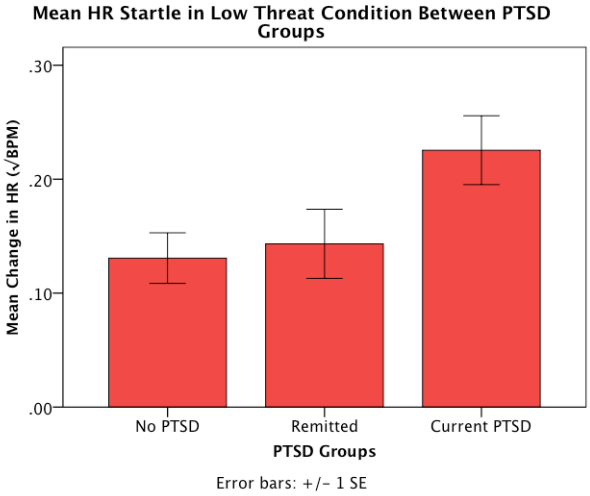
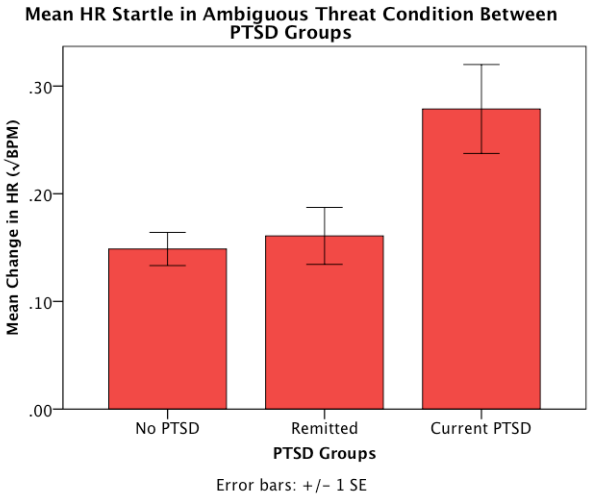
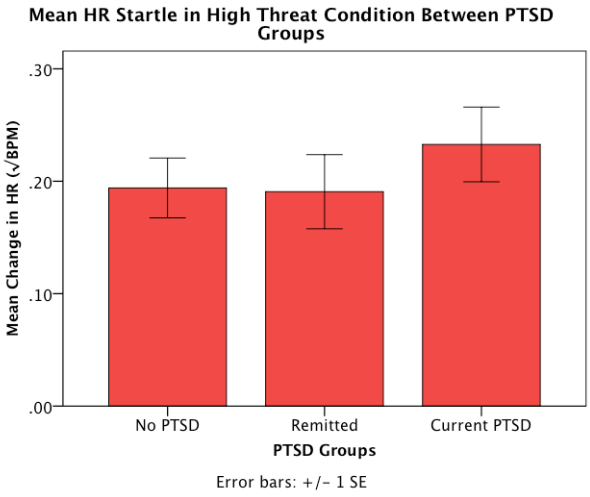
Appendix

Appendix Figure 1: Mean HR Startle at Each Threat Level Across all Groups







Appendix Figure 2: Correlation between Number of Trauma Categories and HR Startle Response