Title
REM Sleep, Safety Signal Learning, and Extinction Processes in Posttraumatic Stress Disorder

Permalink
https://escholarship.org/uc/item/5nc618b9

Author
Straus, Laura Darleen

Publication Date
2017

Peer reviewed|Thesis/dissertation
REM Sleep, Safety Signal Learning, and Extinction Processes in Posttraumatic Stress Disorder

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Clinical Psychology by Laura Darleen Straus

Committee in charge:

University of California, San Diego

Professor Sonya B. Norman, Chair
Professor Sean P.A. Drummond, Co-Chair
Professor Victoria B. Risbrough

San Diego State University

Professor Nader Amir
Professor Vanessa L. Malcarne

2017
The Dissertation of Laura Darleen Straus is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Co-Chair

Chair

University of California, San Diego

San Diego State University

2017
# Table of Contents

Signature Page .............................................................................................................. iii
Table of Contents ........................................................................................................ iv
List of Tables ................................................................................................................ vi
List of Figures ................................................................................................................ vii
Acknowledgements ..................................................................................................... viii
Vita ................................................................................................................................. ix
Abstract of the Dissertation ......................................................................................... x

Chapter 1. Introduction ............................................................................................... 1
  1.1 Fear Conditioning, Safety Learning, and Extinction Processes Are Implicated in
      PTSD ......................................................................................................................... 2
  1.2 Rapid Eye Movement (REM) Sleep is Disrupted in Patients with PTSD .......... 7
  1.3 Neural Mechanisms of Fear, Safety, and Extinction Processes and
      Hypothesized Relationship with Disrupted Sleep ............................................... 8
  1.4 REM Sleep Disturbance is Related to Safety Signal Learning and Extinction
      Processes .................................................................................................................. 9
  1.5 Specific Aims ......................................................................................................... 12

Chapter 2. Methods ...................................................................................................... 14
  2.1 Overview of proposed study ................................................................................ 14
  2.2 Participants .......................................................................................................... 14
  2.3 Procedures .......................................................................................................... 17
  2.4 Measures ............................................................................................................. 21
  2.5 Analytic plan ....................................................................................................... 24
  2.6 Hypotheses .......................................................................................................... 26

Chapter 3. Results ........................................................................................................ 28
  3.1 Participant Demographics .................................................................................. 28
  3.2 Clinical Symptoms ............................................................................................. 28
  3.3 Sleep Summary Data ........................................................................................... 29
  3.4 Fear Conditioning and Extinction Testing ............................................................ 30
  3.5 Safety Learning, Extinction, and REM Sleep Analyses ...................................... 33

Chapter 4. Discussion .................................................................................................. 37
LIST OF TABLES

Table 1. Demographics of the study sample .......................................................... 47
Table 2. Clinical characteristics of the study sample ............................................. 48
Table 3. Sleep Summary Data ............................................................................. 49
Table 4. Safety Learning and Subsequent REM Sleep Consolidation ................. 51
Table 5. REM Sleep Consolidation and Subsequent Fear and Safety Recall ....... 52
LIST OF FIGURES

Figure 1. Explanatory Model................................................................. 53
Figure 2. Timeline of Testing Phase......................................................... 54
Figure 3. Summary of Recruitment and Enrollment.................................... 55
Figure 4. Fear Potentiated Startle Testing Procedures ................................ 56
Figure 5. Fear Acquisition Session.......................................................... 57
Figure 6. Extinction Learning Session....................................................... 58
Figure 7. Extinction Recall Session.......................................................... 59
ACKNOWLEDGEMENTS

Chapters 1 through 4 of this dissertation will be submitted for publication with co-authors, Norman, Sonya; Drummond, Sean; Risbrough, Victoria. The dissertation author was the primary investigator and will be the primary author on this publication.
VITA

2008  Bachelor of Arts, University of California, Berkeley
2008-2011  Research Assistant, San Francisco VA Medical Center
2011-2016  Graduate Student Researcher, University of California, San Diego
2014  Master of Science, San Diego State University
2016-2017  Psychology Intern, San Francisco VA Medical Center
2017  Doctor of Philosophy, San Diego State University/University of California
ABSTRACT OF THE DISSERTATION

REM Sleep, Safety Signal Learning, and Extinction Processes in Posttraumatic Stress Disorder

by

Laura Darleen Straus

Doctor of Philosophy in Clinical Psychology
University of California, San Diego, 2016
San Diego State University, 2016

Professor Sonya B. Norman, Chair
Professor Sean P.A. Drummond, Co-Chair

Posttraumatic Stress Disorder (PTSD) is associated with a number of negative physical and mental health consequences. Fear conditioning plays an important mechanistic role in PTSD, and PTSD patients also show deficits in safety signal learning. Sleep, particularly REM sleep, serves an important role in safety learning and extinction processes in animal models and healthy humans. Nothing is known about the link between REM sleep and safety signal learning or extinction memory in clinical populations.

This study examined the relationship between REM sleep, safety signal learning, and extinction processes in veterans with PTSD (n = 13). Patients’ overnight sleep was characterized in the lab via polysomnography (PSG). The next day, participants underwent a fear conditioning paradigm during which they acquired fear
toward a visual cue. This testing session also included a visual cue that became a safety signal (CS-). Following this, the veterans’ sleep was monitored overnight again, after which they underwent an extinction learning session. Following a third night of sleep, extinction recall was tested. Bivariate correlations examined the relationship between the slope of safety signal learning and subsequent REM sleep consolidation, as well as the relationship between REM sleep consolidation and subsequent extinction recall on the last day of testing.

Results suggest veterans learned to differentiate the CS+ and the CS- on the first day of testing. Veterans who underwent safety learning more quickly on the first day of testing showed more efficient REM sleep that night ($r = .607, p = .028$). On the second day of testing, the patients successfully underwent extinction learning. Patients with a higher percentage of REM sleep on the last night of the study showed less anxious responding to the CS- early on the last day of testing ($r = .688, p = .009$).

PTSD patients who demonstrated better safety learning showed more consolidated REM sleep, which in turn was associated with better subsequent safety re-learning. These results provide additional evidence suggesting REM sleep plays a mechanistic role in the maintenance of PTSD and thus identify a modifiable biological process to target in treatment of PTSD.
CHAPTER 1. INTRODUCTION

Epidemiological studies suggest more than two thirds of people in the general population experience at least one traumatic event over the course of their lifetime (Breslau et al., 1998). A portion of these people go on to develop Posttraumatic Stress Disorder (PTSD). PTSD is characterized by four clusters of symptoms: 1) re-experiencing the traumatic event in the form of intrusive thoughts, fearful reactions to reminders of the event, distressing dreams, and/or dissociative flashbacks; 2) avoidance of internal and external cues associated with the traumatic event; 3) negative alterations in mood and/or negative cognitions about the self, others, and the world; and 4) hyperarousal, experienced as being watchful for threats, difficulty concentrating, irritability, exaggerated startle responses, and difficulty falling or staying asleep (American Psychiatric Association, 2013).

Lifetime prevalence of PTSD is estimated to be approximately 7% in community samples (Kessler, Chiu, Demler, Merikangas, & Walters, 2005), with especially high prevalence rates in victims of interpersonal violence (Resnick, Kilpatrick, Dansky, Saunders, & Best, 1993) and combat veterans (Weiss et al., 1992). PTSD is associated with negative psychiatric outcomes such as increased suicidality (Davidson, Hughes, Blazer, & George, 1991), alcohol abuse and dependence (Stewart, 1996), multiple comorbidities (Kilpatrick et al., 2003), functional impairment (Kessler, 2000), and low quality of life (Mendlowicz & Stein, 2014). Although evidence-based pharmacological (Brady et al., 2000) and psychosocial (Foa, Hembree, & Rothbaum, 2007; Resick & Schnicke, 1992) interventions have been developed for PTSD, not all patients respond to treatment. With regard to gold-standard psychotherapies for PTSD, it is not uncommon to observe nonresponse
rates as high as 50% (Schottenbauer, Glass, Arnkoff, Tendick, & Gray, 2008). It is important to understand the basic biological mechanisms involved in the development and maintenance of PTSD, as well as the mechanisms involved in treatment response, in order to better predict and understand who may not respond optimally to treatment. Additionally, understanding mechanisms that underlie PTSD symptoms will inform development of more effective interventions. This study investigated two such mechanisms: impaired safety signal learning and impaired extinction memory.

Fear Conditioning, Safety Learning, and Extinction Processes Are Implicated in PTSD

Fear conditioning has been hypothesized to play a role in the development and maintenance of PTSD. Patients with PTSD experience intense fear reactions to cues associated with a traumatic event, which provoke strong avoidance to these cues even long after the trauma (American Psychiatric Association, 2013). For example, a victim of sexual assault may experience intense anxiety when encountering someone new who physically resembles the perpetrator, or an Iraq war veteran who was exposed to an IED blast may experience anxiety when seeing debris on the side of the road and make the decision to change lanes to avoid it. Additionally, patients with PTSD have difficulty differentiating between dangerous and safe situations, leading to overgeneralization of avoidance and hyperarousal symptoms (Jovanovic, Kazama, Bachevalier, & Davis, 2012). For example, a combat veteran who experienced an insurgent attack while in a crowded market may avoid crowds or be watchful or “on guard” while in crowds, even after coming home from deployment. During natural recovery from trauma, fear reactions to threat cues are often reduced through extinction learning and development of extinction memories.
(Rothbaum & Davis, 2003). Repeated exposure to these cues in a safe setting allows them to lose their predictive quality for danger (Myers & Davis, 2007; Quirk & Mueller, 2008).

It is hypothesized that patients with PTSD have impairments in fear conditioning and extinction learning processes. Researchers have used classical Pavlovian conditioning paradigms to demonstrate this in the laboratory setting. In these studies, animal or human subjects are exposed to neutral cues which are repeatedly paired with an aversive stimulus (unconditioned stimulus), such as a shock or an air puff to the throat (Lonsdorf et al., 2017). Over time, subjects learn to associate the neutral stimulus with threat and become anxiously reactive in response to the stimulus alone (conditioned stimulus, or CS+). In addition to the threat signal, fear conditioning experiments often present a second neutral stimulus which is never paired with the unconditioned stimulus, so participants learn to associate this cue with safety (CS-, Jovanovic et al., 2010). During extinction learning sessions, the CS+ is then presented repeatedly without being paired with the unconditioned stimulus. Participants undergo extinction by forming new memories allowing for inhibition of the fear response, and thus they no longer respond anxiously in the presence of the CS+.

Finally, some experiments (e.g., Milad et al., 2007) also test recall of extinction by presenting the CS+ during a later testing session and examine whether or not extinction learning is retained over time.

Laboratory studies using these experimental paradigms with PTSD patients have shown abnormalities in both fear conditioning and extinction learning. For example, Grillon and Morgan (1999) showed Gulf War veterans with PTSD were more reactive than healthy controls to a startle probe at baseline. Orr and colleagues
(2000) demonstrated patients with PTSD more readily formed fear associations as compared to trauma-exposed controls, and continued to respond anxiously to the CS+ during extinction trials, suggesting that PTSD patients take longer to “un-learn” fear associations than trauma-exposed control participants. In another laboratory study comparing PTSD patients to trauma-exposed controls, both groups successfully learned to respond anxiously to the CS+ during fear conditioning, but only the PTSD patients were more likely to show impairments in extinction learning (Wessa & Flor, 2007). In addition to research suggesting PTSD patients show enhanced fear conditioning responses and impaired extinction learning, studies have also shown PTSD patients show difficulties with retaining extinction learning over time. In a study of monozygotic twins discordant for combat exposure, Milad and colleagues (2008) demonstrated combat veterans with PTSD showed impairments in extinction recall in comparison to their monozygotic twins without PTSD. Although there were no group differences during an extinction learning session, only participants with PTSD were more likely to show a return of the fear response even after undergoing extinction (Milad et al., 2008). These difficulties with extinction learning have been linked to re-experiencing symptoms of PTSD. PTSD patients appear to have more difficulty learning that cues previously associated with traumatic events (e.g., helicopter sounds for a Vietnam veteran, seeing trash on the side of the road for an Operation Iraqi Freedom veteran) are no longer signals for danger outside of the context of the traumatic event. These cues then continue to provoke intense fear reactions, even when they have been repeatedly encountered in a safe setting (Mineka & Zinbarg, 2006; Rothbaum & Davis, 2003).
In addition to dysregulation in the fear response, it is hypothesized that PTSD patients also show impairments in safety learning, and/or the ability to differentiate between threatening and safe cues. This has been demonstrated in laboratory experiments with fear conditioning paradigms incorporating a conditioned stimulus as well as a separate neutral stimulus (or CS-), which is never paired with the unconditioned stimulus and thus signals safety. For example, Grillon and Morgan (1999) demonstrated that PTSD patients are less able to differentiate threat cues from safety cues during a fear conditioning session, showing anxious responding to cues regardless of whether they were paired with the unconditioned stimulus or not. In a study comparing patients with PTSD to patients with Major Depressive Disorder (MDD), PTSD patients showed difficulty with safety learning, whereas depressed patients did not (Jovanovich et al., 2010). In a large cohort study of US Marines comparing PTSD patients, patients with other anxiety disorders, depressed patients, and controls, the PTSD symptom group was the only group to show deficient discrimination between the CS+ and CS-, exhibiting larger startle responses during the safety cue during fear acquisition (Acheson et al., 2015). All of these studies suggest that in addition to impairments in fear extinction and retention, PTSD patients also have difficulty discriminating between cues signaling threat versus cues signaling safety. Jovanovic and colleagues (2012) hypothesize that difficulties with safety learning are specific for PTSD. In addition to abnormalities with extinction processes, these difficulties with safety learning are also hypothesized to underlie PTSD symptoms. PTSD patients often have difficulty discriminating between threatening and safe environments, leading to overgeneralization of avoidance to both
threatening and safe environments as well as hypervigilance symptoms (Acheson, Gresack, & Risbrough, 2012).

Abnormalities in fear extinction and safety learning processes are not only thought to underlie PTSD symptoms; both safety learning and extinction processes are critical for response to evidence-based psychosocial interventions for PTSD. Prolonged Exposure, the gold-standard behavioral PTSD treatment, involves exposing patients repeatedly to feared cues in a safe environment (Foa, Hembree, & Rothbaum, 2007). For such treatment to be successful, it is necessary for patients to be able to distinguish between threatening and safe cues. Patients must also successfully undergo extinction learning by learning that these trauma-related cues no longer signal the presence of danger. Finally, patients must also retain extinction learning over time in order to maintain the gains of the treatment long-term. Although this process occurs successfully in many patients, not all patients respond, or respond completely, to treatment (Schottenbauer et al., 2008). This variability in treatment response may reflect individual vulnerabilities that impair safety learning and/or extinction processes. Indeed, in a recent study, individuals with social anxiety who demonstrated better extinction learning during a fear conditioning paradigm at baseline reported greater anxiety reduction following brief exposure therapy (Ball, Knapp, Paulus, & Stein, 2016). These findings may apply more broadly to other anxiety and/or trauma-related disorders for which exposure therapy is the gold-standard treatment approach. Thus, safety learning and/or extinction processes represent an important biomarker; examining factors, especially modifiable factors, that influence extinction and/or safety learning processes will identify important mechanisms to target in PTSD treatment. This study examined one such factor, sleep
disturbance, and Rapid Eye Movement (REM) sleep disturbance in particular, as a potential mechanism underlying impaired safety learning, extinction learning, and extinction recall in PTSD.

**Rapid Eye Movement (REM) Sleep is Disrupted in Patients with PTSD**

Some of the most ubiquitous symptoms in PTSD are insomnia and nightmares (Neylan et al., 1998). As many as 90% of veterans with PTSD experience significant regular sleep disturbances (Weiss et al., 1992). Military personnel serving in a combat zone are often sleep deficient, and sleep disruption has been shown to be associated with the emergence of trauma-related mental health symptoms in this population (Taylor et al., 2014). Longitudinal studies with military personnel suggest sleep problems are also the most commonly reported difficulties following deployment (McLay, Klam, & Volkert, 2010), and are associated with the later development of psychopathology, including PTSD specifically (Babson & Feldner, 2010). Though previously thought to be “secondary” to other PTSD symptoms, recent reviews suggest sleep disturbance is a core feature of the disorder (Germain, Buysse, & Nofzinger, 2008; Germain, 2013; Spoormaker & Montgomery, 2008).

REM sleep in particular is implicated in PTSD, in part because most nightmares occur during REM sleep. Studies using polysomnography to measure sleep architecture in PTSD as compared to control participants do not always show consistent differences between groups (see Germain, 2013, for a brief review of this literature), perhaps in part because sleep in PTSD differs widely from individual to individual and from night to night (Straus, Drummond, Nappi, Jenkins, & Norman, 2015). However, these types of studies do often show abnormalities in REM sleep in PTSD patients in comparison to controls or other clinical groups (Kobayashi, Boarts,
& Delahanty, 2007). For example, a study comparing combat veterans with PTSD to patients with depression and a third group of healthy controls found that the PTSD patients showed reduced REM relative to the other two groups (Mellman, Nolan, Hebding, Kulick-Bell, & Dominguez, 1997). Other studies have shown abnormalities in REM density, or the frequency of rapid eye movements during REM sleep (Mellman, Bustamante, Fins, Pigeon, & Nolan, 2002). Additionally, a growing body of research suggests REM sleep fragmentation may be a more consistently observed biomarker of PTSD than total REM duration. In one study, REM sleep fragmentation, as measured by short REM sleep segment duration, was associated with the severity of PTSD symptoms in the early aftermath of trauma (Mellman, Pigeon, Nowell, & Nolan, 2007). Sleep disruption has also been shown to be a risk factor for developing PTSD following trauma exposure (Babson & Feldner, 2010), underscoring the importance of REM sleep as a mechanism involved in PTSD.

**Neural Mechanisms of Fear, Safety, and Extinction Processes and Hypothesized Relationship with Disrupted Sleep**

Despite these findings for a role of sleep in PTSD, the mechanism by which sleep, and REM sleep specifically, influences other PTSD symptoms is unknown. We hypothesize that mechanism is a relationship between REM sleep and both safety learning and extinction processes. Studies examining biological substrates, including brain networks and neurotransmitters involved in fear conditioning and safety signal learning, provide evidence for a connection to disrupted REM sleep. Fear conditioning involves activation of the limbic system and amygdala in particular (Phillips & LeDoux, 1992). Extinction and safety learning, by contrast, have been hypothesized to be a “top-down” process involving frontal regions, such as the
ventromedial prefrontal cortex (vmPFC), to inhibit limbic fear response (Jovanovic & Norrholm, 2011). Fear conditioning also involves activation of adrenergic neurons in the locus coeruleus (LC), which results in elevated norepinephrine (NE; Tanaka, Yoshida, Emoto, & Ishii, 2000) and release of corticotropin releasing factor (CRF; Heinrichs & Koob, 2004). This in turn may fragment REM sleep and potentially leads to nighttime PTSD symptoms, such as nightmares (Raskind et al., 2007). Fragmented REM sleep then subsequently interferes with extinction processes, so conditioned responses to trauma cues are perpetuated long-term via re-experiencing symptoms of PTSD. Though previous studies have found direct associations between REM sleep disturbance, safety learning, and extinction processes (see below), we examined that relationship for the first time in PTSD, here.

**REM Sleep Disturbance is Related to Safety Signal Learning and Extinction Processes**

See Figure 1 for a conceptual model of the link between REM sleep disturbance and PTSD development, maintenance, and response to exposure-based treatment. Briefly, in the model, an acute stress response may be associated with safety learning impairment, which leads to REM fragmentation. REM fragmentation in turn impairs extinction processes, particularly extinction recall. This whole process makes the development of PTSD more likely. PTSD, in turn, is associated with both safety learning impairment and REM sleep disturbance, which would be expected to perpetuate or increase extinction recall impairment, thereby exacerbating daytime symptoms and/or reducing the treatment response to exposure based interventions. We tested the hypothesized links between safety learning, REM sleep, and extinction memory (yellow boxes) in this study. Future studies would test the model's
assumption that treating sleep prior to exposure-based PTSD treatment would facilitate response to such interventions.

Research findings from animal models and healthy humans corroborate the links we have hypothesized in this model. For example, in a study conducted in our research group (Marshall, Acheson, Risbrough, Straus, & Drummond, 2014), 42 healthy control participants underwent a fear conditioning/safety learning paradigm and then their sleep was monitored overnight in the laboratory prior to undergoing extinction learning. Increased safety signal learning during the acquisition phase of the study was associated with more consolidated REM sleep that night. More consolidated REM sleep, in turn, was then associated with better discrimination between the CS+ and CS- early in the extinction learning session 24 hours later. This study, which was conducted in healthy human control participants, presents evidence for the logical inverse of the model proposed here, which hypothesizes that impaired safety learning is associated with more fragmented REM sleep, which is then subsequently associated with impairments in discrimination between threat and safety cues. In another study (Menz, Rihm, & Buchel, 2016), healthy human participants underwent a fear and safety acquisition session, and then a split-night protocol was used to randomize participants to have a larger or smaller proportion of REM sleep the next night and prior to extinction learning (sleeping the first half of the night leads to relatively less REM sleep and sleeping only the second half of the night leads to more REM). Following a night of recovery sleep, participants randomized to have disrupted REM sleep showed worse discrimination of threat and safety signals. This study provides additional evidence in support of our model of PTSD, which
postulates that impaired REM sleep is associated with worse ability to discriminate between threatening and safe cues.

In addition to studies demonstrating links between sleep and safety learning, additional research implicates sleep as a critical factor in fear extinction processes. In animal models, sleep deprivation interferes with extinction consolidation (Hussaini, Bogusch, Landgraf, & Menzel, 2009), and REM sleep deprivation in particular impairs recall of extinction learning (Fu et al., 2007; Datta & O’Malley, 2013). Similar findings have been demonstrated in healthy human control participants. In one study (Pace-Schott et al., 2009), participants were randomized to two different conditions: 1) extinction learning in the evening with an extinction recall session in the morning following a normal night of sleep, or 2) extinction learning in the morning and then recall testing in the evening, without sleep in between. These researchers found participants in the sleep condition were better able to generalize fear extinction than participants who remained awake between extinction learning and recall. In another study (Spoormaker et al., 2010), participants underwent extinction learning and then were allowed to take a nap prior to extinction recall testing. Participants were divided into two categories: 1) those who showed REM sleep during the nap, and 2) those who did not. Those participants who had REM sleep showed better extinction recall than those without REM. In an additional study in healthy human control subjects by the same research group (Spoormaker et al., 2012), participants underwent fear conditioning and extinction learning, and were then randomized to normal sleep or REM deprivation overnight prior to an extinction recall session. REM-deprived participants demonstrated impairments in extinction recall relative to participants who slept normally. In another manuscript based on our group’s study cited above, 71
healthy human control participants underwent a three-day fear acquisition, extinction learning, and extinction recall paradigm. Participants were randomized to one of three groups: 1) normal sleep throughout the study (n = 21), 2) total sleep deprivation for one night prior to extinction learning (n = 25), and 3) total sleep deprivation for one night prior to extinction recall (n = 25). Participants who underwent total sleep deprivation prior to extinction learning demonstrated normal extinction learning but impaired recall of extinction one day later (Straus, Acheson, Risbrough, & Drummond, 2017). Additionally, REM sleep consolidation was correlated with extinction recall on the final day of testing. These studies, though conducted in healthy human control subjects, suggest sleep, and REM sleep in particular, is critical for extinction processes, particularly extinction recall.

**Specific Aims**

The research cited above, conducted in animals and healthy human subjects, provides evidence for our theoretical model (Figure 1) by demonstrating that impaired safety learning is associated with REM sleep fragmentation, which then leads to subsequent difficulties discriminating between threat and safety cues. These studies also show that disrupted sleep, and fragmented REM sleep in particular, interferes with extinction processes, especially extinction recall. Despite these prior studies, no studies have investigated the role of REM sleep in safety learning or extinction processes in PTSD patients. Such an investigation is a critical translational step in demonstrating that sleep disturbance is a potential mechanism underlying PTSD symptom development, maintenance, and recovery. The current study was first to examine the relationship between REM sleep, safety signal learning, and extinction recall in PTSD patients. See Figure 2 for a study timeline. Patients’ overnight sleep
was characterized in the lab. The next day, they underwent a fear conditioning paradigm that included learning of safety signals, followed by a second night in the sleep lab. The next day, participants underwent an extinction learning session. Following this, their sleep was monitored overnight again, after which extinction recall was tested on the last day of the study. Analyses examined the relationship between safety signal learning and subsequent changes in REM sleep, as well as the relationship between REM sleep and next-day safety retention and extinction recall. We hypothesized impaired safety signal learning would be associated with greater fragmentation of REM sleep overnight. Additionally, we hypothesized greater REM fragmentation would be associated with more impaired extinction recall at a subsequent testing session. If our hypotheses are borne out, they will suggest REM sleep plays a mechanistic role in the maintenance of PTSD and will thus identify a modifiable biological process to target in treatment of PTSD.

**Aim 1:** Examine if impaired safety signal learning is related to subsequent REM sleep fragmentation in patients with PTSD.

*Hypothesis 1:* Impaired safety signal learning will be associated with greater subsequent REM sleep fragmentation in patients with PTSD.

**Aim 2:** Examine if REM sleep fragmentation is related to impaired extinction recall in patients with PTSD.

*Hypothesis 2:* Greater REM sleep fragmentation will be associated with decreased extinction recall in patients with PTSD.

Sections of Chapter 1 of this dissertation will be submitted for publication with co-authors, Norman, Sonya; Drummond, Sean; Risbrough, Victoria. The dissertation author was the primary investigator and will be the primary author on this publication.
CHAPTER 2. METHODS

Overview of Proposed Study

The PI of this study recruited all participants and collected or oversaw collection of all study data at the Laboratory for Sleep and Behavioral Neuroscience at the VA San Diego Healthcare System (VASDHS) with the support of an individual predoctoral research fellowship from the NIMH (F31MH106209). All participants were recruited through the VASDHS and signed informed consent between August 6th, 2015 and June 1st, 2016. The VA Internal Review Board as well as the University of California, San Diego’s Human Research Protections Program approved all recruitment, consent and testing materials for this study. Twenty-six participants enrolled in the study and underwent an eligibility visit to establish that they met inclusion and exclusion criteria. Those who were eligible to participate (n = 13) underwent one week of baseline sleep assessment and then spent three nights in the laboratory. Participants’ sleep was monitored overnight, and they also underwent a three-day fear conditioning/safety learning, extinction, and extinction recall sessions. The overall aim of the study was to examine relationships between fear conditioning/safety learning and overnight REM sleep fragmentation, and to examine REM sleep fragmentation and its relationship with subsequent extinction processes.

Participants

Participants consisted of veterans diagnosed with PTSD. We chose to limit the population studied to veterans because: 1) veterans show especially high rates of PTSD (Weiss et al., 1992); therefore, our study’s results should generalize to this critical population; and 2) our research group has a history of recruiting veterans with PTSD for past research studies and has established relationships with internal VA...
referral sources. Payment was provided for in-person eligibility screening as well as participation in the testing phase of the study (see below).

**Recruitment:** Participants were recruited through a number of different methods. These included: 1) coordinating with the PTSD clinical team at the La Jolla VA Medical Center and calling patients on the waiting list for individual therapy to offer them the option to participate in the study, 2) coordinating with other research teams at the La Jolla VA Medical Center to offer them the option of participating in the current study in addition to other assessment studies with similar inclusion/exclusion criteria, and 3) distributing flyers throughout the medical center and broadcasting advertisements on VA TV screens located in clinic waiting areas.

**Inclusion criteria:** 1) Veteran with a primary diagnosis of PTSD; 2) 18 to 50 years old; and 3) literate in English.

**Exclusion criteria:** 1) A history of mania and/or psychosis; 2) substance use disorder during the prior 6 months; 3) diagnosed (previously or by our study screen) and untreated sleep disorder other than insomnia and nightmares; 4) change in type and/or dosage of psychotropic medication in preceding 2 months; 5) history of severe TBI.

**Justification for inclusion/exclusion criteria:** Inclusion and exclusion criteria were chosen to minimize risks and confounds associated with sleep assessment and/or the behavioral paradigms (i.e., high internal validity) while maintaining as much generalizability as possible (i.e., high external validity). Comorbid mood and anxiety disorders were expected and permitted (to maximize generalizability) if PTSD symptoms were judged to be predominant based on primacy and severity of symptoms. We considered expanding inclusion criteria to veterans of all ages.
However, we decided against it due to aging-related changes in sleep architecture that occur after age 50 (Ohayon, Carskadon, Guilleminault, & Vitiello, 2004). We originally considered excluding veterans taking hypnotic medications due complications when considering multiple different hypnotics. For example, benzodiazepines and trazodone may suppress REM (Borbély et al., 1985), while prazosin may enhance REM (Pellejero, 1984). Additionally, use of benzodiazepines and/or prazosin may actually inhibit extinction learning (Bouton, et al., 1990), which would mask the hypothesized relationship between sleep and fear potentiated startle parameters. We considered that disallowing use of hypnotics for the purposes of this study will serve as a more “pure” test of these biological processes, thus favoring internal over external validity. After much consideration, we decided to include veterans taking hypnotics in our study. Our rationale for this was: a) a large portion of veterans in treatment for PTSD at the VA are prescribed hypnotics (Mohamed & Rosenheck, 2008), so including veterans on hypnotics would enhance generalizability of the study and also aid in meeting recruitment goals; and b) including patients on hypnotics increases variability of REM sleep and thus be desirable for our analyses. Overall, we decided to allow veterans prescribed trazodone, zolpidem, and/or prazosin to participate in the study, though we ultimately decided to disallow benzodiazepines due to the medication’s blunting effect on fear conditioning and extinction learning parameters.

**Compensation:** Compensation was given as follows: $10 for the initial in-person assessment, $20 for the week of sleep diary and wearing the actigraph (see below for procedures), $50 for Night 1, $100 for Night 2 and for completing the first
day of in-lab testing, $120 for Night 3 and for completing the second day of in-lab testing. Participants who completed the entire study received a total of $300.

**Summary of Recruitment/Enrollment:** See Figure 3 for a breakdown of recruitment and enrollment in the current study. The PI of this study reached out to a total of 100 veterans to offer them the opportunity to participate. Of these, 44 phone screens were conducted, which yielded 26 baseline assessment visits. Of these, 6 participants (23.1%) were ineligible due to being non-responders on the startle threshold testing (see below). Five participants (19.2%) voluntarily withdrew from the study prior to the sleep lab phase (one was unable to tolerate the startle testing, one had concerns about the reimbursement, one had schedule conflicts, and two were lost to follow-up). Therefore, 15 participants were enrolled and participated in at least one in-lab sleep study. One veteran was dropped after Night 1 because his baseline sleep study revealed that he had severe untreated sleep apnea, and one additional participant dropped out of the study after Night 2 due to feeling ill. Therefore, the total yield was 13 participants with complete data sets.

**Procedures**

**Eligibility Phase:** Initial screening of participants took place via telephone. Participants were given an overview of the study and, after providing verbal consent, were screened with a questionnaire covering the major inclusion/exclusion criteria. Those not excluded for obvious violations of eligibility (e.g., outside of the targeted age range) received an in-person screen, including a series of measures to confirm eligibility (see **Measures** below). Informed consent was signed at the beginning of this first in-person meeting. Participants also underwent a **Startle Threshold Testing** session, which consisted of presentation of 16 startle probes (in the absence of any
visual cue) to examine baseline habituation. Veterans who did not meet the criterion of responding to at least 75% (12 of 16) of the startle probes were deemed nonresponders and excluded from participation in the study.

Those not excluded based on these measures underwent one week of baseline sleep assessment at home, during which they filled out Sleep Diaries and wore a wrist actigraph to characterize baseline sleep.

**Testing Phase:** After the baseline week, participants were brought to the Laboratory for Sleep and Behavioral Neuroscience, where they spent three consecutive nights with their sleep monitored by polysomnography. They also underwent a conditioning paradigm with established reliability in the literature (Davis, Antoniadis, Amaral, & Winslow, 2008; Grillon, 2008). See Figure 2 below for study timeline. Participants underwent an adaptation night (Night 1) to adjust to sleeping in the laboratory environment. This night was also used to screen for exclusionary sleep disorders (e.g., sleep apnea). Night 1 was followed by **Fear/Safety Acquisition** (described below) on Day 1. After a second night in the laboratory (Night 2), participants underwent **Extinction Learning** on Day 2. Participants then spent a third night of sleep in the laboratory (Night 3), followed by **Extinction Recall** testing on Day 3. See Figure 4 for a diagram showing the FPS testing procedures for each session.

**Fear/Safety Acquisition**: Testing sessions were conducted 1 hour following participants’ habitual wake time. For all sessions, participants were seated in a lounge chair in a sound-attenuated room. Visual cues were presented via LCD monitor. Sensors were fitted to measure Blink Reflex, was used as operational
measures of the conditioned fear response, safety learning, extinction, and extinction recall.

During this phase, participants underwent a procedure to acquire conditioned fear a visual cue (the conditioned stimulus, or CS+). This fear potentiated startle (FPS) paradigm has been used for several research studies conducted at the Psychophysiology Unit of the Center of Excellence for Stress and Mental Health at the VASDHS (Dr. Risbrough, Director), after being established by Norrholm and colleagues (Norrholm et al., 2011; Norrholm et al., 2013; Norrholm et al., 2006). We conducted FPS acquisition and extinction as previously described (Acheson et al., 2013). In brief, the unconditioned stimulus (US) was a puff of air delivered to the throat. FPS acquisition began with an acclimation period during which 70 db of broad band background noise was be played through the participants’ headphones, followed by six 108 db, 40 ms acoustic startle probes. Following the acclimation period, 4 trial types were presented: 3 conditioned stimulus (CS) trials and one no-stimulus trial. For each CS trial, 1 of 2 possible shapes were presented on the LCD monitor, for 6 seconds. Between 4-5 seconds after the onset of the shape, a startle probe was delivered to assess fear conditioning. One of these shapes (a blue circle, serving as CS+) was paired with an air puff to the throat 75% of the time. The second shape (yellow circle) was never paired with the air puff and thus served as the safety signal (CS-). Participants were not explicitly told about the predictive quality of these visual cues, but participants reported air puff expectancy (“yes,” “no,” or “unsure”) for each trial via a keypad. Overall, the FPS acquisition phase consisted of 24 trials, including 8 CS+ trials, 8 CS- trials, and 8 Noise Alone (NA) trials during which the startle probe was presented in the absence of any visual cue. Stimulus presentation
was block randomized with the constraint of two trials of each type (CS+, CS−, and NA) per block. FPS was measured by eyeblink magnitude in response to the acoustic stimuli presented in the presence/absence of CS+. The degree of relative startle potentiation in the presence of the CS+ during the last two blocks of this phase of testing, compared with absence of any visual cue (i.e., NA trials) was used as an operational measure of fear response.

**Safety Learning** was measured by the calculated difference in CS- blink response from the first block, when subjects typically potentiate to all stimuli before they have learned the contingency, to the second block when CS+/CS- discrimination begins to occur (Marshall et al., 2014, Acheson et al., 2013). With this calculation, large numbers indicate rapid safety learning, as large numbers indicate participants showed a smaller fear response to the CS- at the end of the test session, relative to at the start of the session. In contrast, comparably small numbers indicate impaired safety learning, as small numbers indicate participants were not able to modulate their fear response by the end of the test session, despite multiple presentations of this cue without pairing with the aversive stimulus. It was hypothesized (see **Aim 1**) that impaired safety learning would be associated with more fragmented REM sleep that night (Night 2). After the conclusion of this testing session, participants filled out a questionnaire during which they reported which shape predicted the air puff, which served as a manipulation verification.

**Extinction Learning:** After spending a second night in the laboratory and 24 hours after Fear/Safety Acquisition, participants underwent a standard extinction procedure that consisted of 72 trials (16 CS+, 16 CS-, 16 NA). Visual cues and startle probes were presented as in the Fear/Safety Acquisition phase, but no air puffs were
delivered. The CS+ previously paired with the air puff (the blue circle) was presented during this testing session repeatedly without the air puff. Participants therefore had the opportunity to “un-learn” the association between the CS+ the US (i.e., the conditioned response was extinguished).

**Extinction Recall**: This phase was conducted 24 hours after the Extinction learning session, after Night 3 (see Figure 2). It consisted of 24 trials (8 CS+, 8 CS-, 8 NA). During this session, both the CS+ and CS- were presented repeatedly, and no cues were paired with the air puff. Extinction Recall was operationalized as the average Blink Reflex to the CS+ over the first two blocks of testing, consistent with prior research using this paradigm (Milad et al., 2009). Successful extinction recall corresponded to relatively small physiological responses to the CS+ during this testing phase in comparison to the participant’s own baseline (as participants learned during the previous testing phase that the CS+ is no longer paired with the US). However, failure to retain extinction learning resulted in comparably large physiological responses to the CS+ during this phase of testing (suggesting participants did not retain knowledge the CS+ is now safe). It was hypothesized (see **Aim 2**) that more fragmented REM sleep on Night 3 would correspond to decreased extinction recall on Day 3 during this phase of testing.

**Measures**

**Eligibility Assessments**:

**Clinician Administered PTSD Scale, DSM-5 version (CAPS-5)**: The CAPS is a semistructured interview corresponding to DSM-5 criteria for PTSD (Weathers, Blake, & Schnurr, 2015). It was recently adapted to correspond to DSM-5 symptoms of PTSD and is widely considered to be the gold standard PTSD assessment.
(Weathers, Keane, & Davidson, 2001). For this study, the CAPS was the primary method used to establish PTSD diagnosis.

**Structured Clinical Interview for DSM-IV (SCID):** The SCID is a clinician-administered diagnostic interview based on DSM-IV criteria (First, Spitzer, Gibbon, & Williams, 2012). For this study, individual modules of the SCID were used to rule out exclusionary Axis I disorders, including psychotic symptoms, manic symptoms, and substance abuse/dependence during the prior 6 months (see above).

**Medications Use Interview:** All participants completed a standard interview regarding recent medication use (developed by the investigators). Additionally, participants were instructed to bring in the names and dosing schedule of all medications they are currently being prescribed (when this was unclear, the PI verified this information using the patient’s medical record in the VA’s Computerized Medical Records System). For this study, this interview was used to rule out participants who had changed the type and/or dosage of psychotropic medication in preceding 2 months, or were taking benzodiazepines (see above). All interviews were conducted by a single study assessor (the PI of this study), who was working under the supervision of Drs. Norman and Drummond. Dr. Norman has particular expertise in PTSD assessment, including the CAPS, while Dr. Drummond has extensive experience with using all of these assessments in previous studies (e.g., NINR #1RC1NR011728). Drs. Norman and Drummond supervised the PI and ensured adherence to standardized assessment procedures.

**Sleep Assessments:**

**Sleep Diaries:** The sleep diary included typical subjective measures (such as bed time, wake time, sleep latency, number and duration of awakenings, total time in
bed) and two calculated variables (total sleep time and sleep efficiency). Frequency
and intensity of nightmares were also assessed. Daily sleep diaries are commonly
used in sleep research (Carney et al., 2012), and we have used this one in several
studies.

**Actigraphy:** Actigraphy is the most commonly used validated objective sleep
assessment outside of a laboratory context (Ancoli-Israel et al., 2003). Respironics
Actiwatch 2 and Actiware software were used to calculate total sleep time, wake after
sleep onset, and sleep efficiency. For this study, Sleep Diaries and actigraphy were
used to assess insomnia symptoms, nightmares, and diurnal rhythms for the at-home
baseline week of the study (see above). These data were not used for investigation of
the main study aims, but instead were used to further characterize sleep in this
sample.

**Polysomnography (PSG):** During the Testing Phase of the study (see
above), participants’ sleep was monitored with a standard overnight polysomnogram,
including EEG, EOG, and EMG. On Night 1, additional monitors screened for sleep
apnea and periodic leg movements. Sleep recordings were scored for sleep stages
(i.e. N1, N2, N3, REM) by a single scorer and according to standardized procedures
(Medicine & Iber, 2007). Our three variables representing REM consolidation (See
Data Analysis below) were determined by this scoring.

**Psychophysiological Assessments:**

**Blink Reflex** was measured during Fear/Safety Acquisition, Extinction
Learning, and Extinction Recall via two small EMG cup electrodes (Ag/AgCl) placed
below and lateral to the left eye over the orbicularis oculi muscle, referenced to an
electrode placed over the left mastoid.
Analytic Plan

Data Analysis and Hypothesis Testing: Descriptive statistics were used to characterize demographics and clinical symptoms (i.e., CAPS, PCL-S, PHQ-9) in the sample of veterans recruited for the study. Descriptive statistics were also used for the Sleep Diary, Actigraphy, and PSG in order to better characterize sleep in the sample.

Prior to data analysis, physiological data was scored according to our research group’s previously-established procedures (see Straus et al., 2017). CS+ and CS- trials were averaged within each session into blocks of two trials each. NA trials within a session were averaged to acquire a baseline startle response. This baseline was then subtracted from the respective CS+ and CS- block within each session, creating scores representing potentiated startle above baseline for each CS type within each block. Thus, there were four blocks for the CS+ and CS- during the acquisition session; eight blocks for the CS+ and CS- for the extinction session; and four blocks for the CS+ and CS- during the recall session. Data were then examined to test statistical assumptions (e.g. normality) and missing data patterns were also investigated. Participants who did not complete all components of the testing phase of the study were dropped from final analyses.

Fear Conditioning, Safety Learning, and Extinction Data Analysis: The Day 1 Fear/Safety Acquisition session was analyzed by using a priori paired samples \( t \)-tests to examine the response to the CS+ at the beginning of the testing session (Block 1) relative to the end of the session (Block 4). Similar paired samples \( t \)-tests were used to compare response to the CS- at the beginning of the session relative to the end. Then, paired samples \( t \)-tests were used to compare the response to the CS+
to the CS- at each block. It was hypothesized participants would be able to distinguish between the threat and safety signal to a greater extent at the end of the testing session in comparison to the beginning. As in our previous work (Straus et al., 2017), for the extinction learning session on Day 2, Blocks 1 and 2, Blocks 3 and 4, Blocks 6 and 6, and Blocks 6 and 7 were averaged together to create variables representing early, middle, and late extinction learning. Then, paired samples \( t \)-tests were used to examine response to the CS+ at the beginning of the testing session relative to the end of the session. Similar paired samples \( t \)-tests were used to compare response to the CS- at the beginning of the session relative to the end. Finally, paired samples \( t \)-tests were used to compare the response to the CS+ to the CS- at each of the time points (early, middle, late). Similar paired samples \( t \)-tests were performed to examine participants’ responses on the Recall session on Day 3.

**REM Sleep and FPS Analyses:** This study aimed to examine the relationship between safety signal learning and subsequent REM consolidation (Aim 1). Additionally, this study aimed to examine the relationship between REM sleep consolidation and subsequent extinction recall (Aim 2). REM consolidation was defined based on three variables: 1) REM Percent (i.e., REM sleep duration divided by total sleep duration), 2) REM Efficiency (i.e., REM minutes divided by the total duration of all REM periods), and 3) REM Latency (i.e., duration of non-REM sleep before the first REM onset). Prior to investigation of the main hypotheses, we created a correlation matrix to examine co-linearity among these three variables by examining Pearson correlation coefficients. If two or more variables appeared to be co-linear
(e.g., $r > .7$), redundant predictors were dropped from the model. Predictors with the highest theoretical importance were retained.

**Hypotheses**

**Hypothesis 1:** Greater levels of safety signal learning will be associated with greater subsequent REM sleep consolidation in patients with PTSD.

To examine the effect of safety learning on subsequent REM sleep consolidation (Aim 1), a difference score was created based on the blink response to the CS- on Block 1 of the Acquisition session in comparison to Block 2 (Block 1 – Block 2) to create a continuous variable representing the slope of safety learning. We predicted that large numbers would indicate rapid safety learning, as large numbers would indicate participants learned to modulate their fear response in response to the CS- after a few trials. Inversely, we predicted that comparably small numbers would indicate impaired safety learning, as small numbers would indicate participants were not able to modulate their fear response despite multiple presentations of this cue without pairing with the aversive stimulus. Bivariate correlation was then used to examine the relationship between this score and REM latency, REM efficiency, and REM percent on the subsequent night (Night 2).

**Hypothesis 2:** Greater levels of REM sleep fragmentation will be associated with decreased extinction recall in patients with PTSD.

To examine the relationship between REM sleep consolidation and Extinction Recall on the final morning of testing (Aim 2), bivariate correlations were used to explore associations between REM efficiency, REM percent and REM latency on Night 3 and response to the CS+ early in the Recall session on Day 3. We predicted that more consolidated REM sleep would be associated with less anxious responding to the
CS+ early in the session, similar to what we have observed in previous studies conducted by our research group (Straus et al., 2017). Additionally, we conducted additional analyses to examine relationships between REM sleep consolidation on Night 2 and Extinction Recall on Day 3, as previous studies have noted that sleep prior to extinction learning is associated with recall of extinction (Straus et al., 2017, Menz, Rihm, & Buchel, 2016). The three measures of REM consolidation from Night 2 were correlated with responses to the CS+ early in the Recall session. We hypothesized that more REM sleep consolidation on Night 2 would be associated with less anxious responding to the CS+ on Day 3 of testing.

Sections of Chapter 2 of this dissertation will be submitted for publication with co-authors, Norman, Sonya; Drummond, Sean; Risbrough, Victoria. The dissertation author was the primary investigator and will be the primary author on this publication.
CHAPTER 3. RESULTS

Participant Demographics:

See Table 1 for demographics of the study sample. As noted above, 15 participants completed at least one night of the testing phase of the study, which yielded 13 veterans with complete data sets. Of the participants who completed the entire study, the average age was 31.88 with a standard deviation of 7.67. The youngest participant was 25, and the oldest was 42. Eleven out of the thirteen veterans were male (84.6%) and two were female (15.4%). Eleven (84.6%) were Caucasian, one was African American (7.7%), and one identified as a Pacific Islander (7.7%). Eight participants did not identify as Hispanic (61.5%) and five were Hispanic (38.5%). Overall, 7 of 13 were members of non-majority populations, underscoring the ethnic diversity of this small sample of veterans. Six participants were veterans of the Marine Corps (46.2%), four were veterans of the Army (30.8%), and three were veterans of the Navy (23.1%). Regarding education, the majority had some college education without completing a degree (n = 11, 84.6%). One participant completed a 4-year college degree (7.7%), and one completed an MBA (7.7%).

Clinical Symptoms:

See Table 2 for a summary of the clinical symptoms data obtained in this sample. At the initial visit when participants underwent eligibility assessments including the CAPS, Criterion A traumatic events were identified and classified by type. The majority of these were combat-related events from the recent conflicts in Iraq and Afghanistan (n = 9, 69.2%). Two participants (15.4%) identified Military Sexual Trauma as their Criterion A event. One veteran (7.7%) experienced
combat exposure as well as a motor vehicle accident during his service. One participant (7.7%) identified witnessing a friend’s suicide. On the CAPS, all veterans met criteria for PTSD according to DSM-5 criteria. The average CAPS score was 42.85 (with a standard deviation of 12.22), which is indicative of moderately severe PTSD symptoms. On the PCL-S, the average score was 55.68 (with a standard deviation of 16.29), which is above the clinically significant threshold that has been suggested for veteran populations at VA clinics (Yeager, Magruder, Knapp, Nicholas, Frueh, 2008). In this sample, the average score on the PHQ-9 was 14.31 (with a standard deviation of 6.94), which is indicative of moderately severe depression.

**Sleep Summary Data:**

See Table 3 for a summary of the scores obtained from the sleep measures in this study.

**Questionnaires:** The average score on the ISI was 14.77 (with a standard deviation of 5.63), which is suggestive of moderate insomnia. On the PSQI, the veterans’ average score was 11.86, which is considerably above the suggested threshold of 5 indicating clinically significant sleep disturbance (Buysse et al., 2008). On the PSQI Addendum for PTSD, the average score in this sample was 10.77 (with a standard deviation of 4.59), which is indicative of additional sleep disturbance specifically related to PTSD symptoms (e.g., nightmares, anxiety and hyperarousal at night, “acting out” dreams).

**Sleep Diaries:** For the baseline week, the veterans in this study reported spending just under 8 hours in bed on average with a standard deviation over 60 minutes. Total Sleep Time just under 6 hours, with a standard deviation of approximately 80 minutes. Sleep Latency averaged over 40 minutes, and Wake After
Sleep Onset averaged approximately 78 minutes. Sleep Efficiency averaged approximately 75% for the baseline week with a standard deviation of 12.4%. On the whole, this sample showed clinically significant insomnia symptoms, with a wide variability between participants, replicating previous studies showing high variability of sleep in PTSD patients (Straus et al., 2014).

**Actigraphy:** On actigraphy, the veterans in this sample showed an average of approximately 6 hours of sleep per night on average (with a standard deviation of 43.83 minutes). Wake After Sleep Onset averaged just over 45 minutes per night, and Sleep Efficiency averaged approximately 77%, with a standard deviation of approximately 8%.

**Polysomnography:** The three nights of sleep in the lab were averaged over the three nights to obtain sleep continuity metrics to match those obtained by Sleep Diaries and Actigraphy. On PSG, Total Sleep Time averaged just over 6 hours, with a standard deviation of just under 55 minutes. On average, it took the veterans about 18 minutes to fall asleep, and the veterans showed an average of just under 30 minutes awake in the night, though the standard deviation for this variable was approximately 26 minutes. Mean Sleep Efficiency was just under 90%, with a standard deviation of approximately 6.5%.

**Fear Conditioning and Extinction Testing:**

**Fear Acquisition:** See Figure 4 for a schematic of participants’ responses to the CS+ and CS- over the course of the fear acquisition session. Paired samples t-tests were used to compare the response to the CS+ to the CS- at each block. Paired samples t-tests were then used to examine response to the CS+ at the beginning of the testing session (Block 1) as compared to the end of the session (Block 4). Similar
paired samples t-tests were used to compare response to the CS- at the beginning of the session relative to the end. The participants responded similarly to the CS+ and the CS- during the first block ($t = 0.04, p = .973$), but by the last block they responded more anxiously to the CS+ than they did to the CS- ($t = 2.36, p = .034$). Participants responded more anxiously to the CS+ during the first block of the session than during the last block ($t = 2.43, p = .030$). They also responded more anxiously to the CS- during the first block as compared to the last block ($t = 3.36, p = .005$). So, in summary, participants were more reactive to both stimuli at the beginning of this session than they were at the end, suggesting they habituated to both stimuli to some extent. However, given this, they still responded more anxiously to the CS+ than they did to the CS- by the end of the session, so this suggests they learned to differentiate between the “threat signal” and the “safety signal” overall and responded more anxiously to the threat signal.

**Extinction Learning:** See Figure 5 for a diagram of participants’ responses to the CS+ and CS- over Extinction learning. Paired samples t-tests were used to examine response to each cue at the beginning of the testing session relative to the end of the session. Then, paired samples t-tests were used to compare the response to the CS+ to the CS- at each time point. Results revealed that participants responded more anxiously to the CS+ during early extinction than they did during late extinction ($t = 4.07, p = .002$). They also responded more anxiously to the CS- during the first block as compared to the last block ($t = 5.55, p < .001$). Participants responded more anxiously to the CS+ than they did to the CS- during early extinction learning ($t = 2.26, p = .042$), though by late extinction learning there was no difference in response between the two cues ($t = 1.24, p = .242$). In summary, these results
suggest participants responded less anxiously to both cues over the course of the extinction session, though they responded more anxiously to the CS+ in comparison to the CS- at the beginning of the testing session, likely because they learned that this cue signaled threat in the testing session the day before. By the end of the testing session, however, participants appeared to learn that the “dangerous” cue was now safe, and re-learned that the “safety signal” was still safe, and thus no longer discriminated between the two cues.

**Extinction Recall:** See Figure 6 for a diagram showing participants’ responses to the CS+ and CS- over the Extinction Recall session. Similar to the Acquisition and Extinction learning sessions above, paired samples t-tests were used to examine response to each cue at the beginning of the testing session relative to the end of the session, as well as to compare the response to the CS+ to the CS- at each time point. These tests revealed that participants responded more anxiously to the CS+ during the first block of the session than during the last block ($t = 3.84, p = .003$). They also responded more anxiously to the CS- during the first block as compared to the last block ($t = 2.54, p = .026$). There were no differences between responses to the CS+ and CS-, either in the first block ($t = 1.36, p = .198$) or in the last block ($t = 0.57, p = .581$). Similar to the Extinction session, these results suggest participants responded less anxiously to both cues over the course of this testing session. However, they did not differentiate the CS+ versus the CS- early or late in the testing session. This suggests participants successfully underwent extinction learning on the previous day such that by the Recall session on Day 3 they had already learned that neither cue signified threat and thus responded similarly to both cues.
Safety Learning, Extinction, and REM Sleep Analyses:

Safety and Fear Acquisition and Subsequent REM Sleep: To examine the effect of safety learning on subsequent REM sleep consolidation (Aim 1), a difference score was created (Block 1 – Block 2) to create a continuous variable representing the slope of safety learning. Large numbers represented rapid safety learning, as large numbers indicated participants learned to modulate their fear response in response to the CS- after a few trials. Inversely, comparably small numbers represented impaired safety learning, as small numbers indicated participants were not able to modulate their fear response despite multiple presentations of this cue without pairing with the aversive stimulus. Bivariate correlation was used to examine the relationship between this score and REM latency, REM efficiency, and REM percent on the subsequent night (Night 2). See Table 4 for these results. There was a significant relationship between slope of safety learning and subsequent REM efficiency on Night 2 ($r = .607, p = .028$). Slope of safety learning was not significantly associated with REM latency ($r = .168, p = .583$) or REM percent on Night 2 ($r = .22, p = .474$). A second difference score was created to examine the magnitude of safety learning over the entire testing session (Block 1 – Block 4). This measure was then correlated with the three variables representing REM sleep consolidation. No significant relationships were found (all $p$ values $> .3$).

Though not connected to the main aims of this study, exploratory analyses were conducted to examine the relationship between fear learning and subsequent REM sleep, because previous studies, particularly in the animal literature, suggest that fear conditioning disrupts and fragments subsequent sleep. No relationships were found in correlating magnitude of response to the CS+ late in the fear
acquisition session (when participants were expected to be able to differentiate between threat and safety cues) and subsequent REM latency \( (r = .27, p = .378) \), REM efficiency \( (r = -.36, p = .228) \), or REM percent \( (r = -.08, p = .785) \).

**REM Consolidation and Extinction Learning:** A previous study from our research group has shown that consolidated REM sleep is associated with enhanced ability to discriminate between fear and safety signals in a subsequent testing session (Marshall et al., 2014). This potential relationship was explored in the current study. A difference score \( (CS^+ - CS^-) \) was created to operationalize the ability to discriminate between the threat and safety signal early in the Extinction learning session. Then, bivariate correlations were conducted between the three REM consolidation variables on Night 2 and this measure of fear/safety discriminability. None of these correlations reached significance (REM latency: \( r = -.48 \); REM efficiency: \( r = .04 \); REM percent: \( r = .20 \), all \( p \) values > .08).

**REM Sleep Consolidation and Subsequent Safety/Fear Recall:** To examine the relationship between REM sleep consolidation and Extinction Recall on the final morning of testing (Aim 2), bivariate correlations were used to explore associations between REM efficiency, REM percent and REM latency and response to the CS+ early in the Recall session. We predicted that more consolidated REM sleep would be associated with less anxious responding to the CS+ early in the session, similar to what we have observed in previous studies conducted by our research group (Straus et al., 2017). See Table 5 for these results. Bivariate correlations revealed no significant associations between REM efficiency \( (r = -.19, p = .540) \), REM percent \( (r = .17, p = .587) \), nor REM Latency \( (r = .38, p = .200) \), and anxious responding to the CS+ early in the Recall session.
To explore further potential relationships between prior sleep and extinction recall (Aim 2), we conducted additional analyses to examine relationships between REM sleep consolidation on Night 2 and Extinction Recall on Day 3, as previous studies have noted that sleep prior to extinction learning is associated with later recall of extinction (Straus et al., 2017, Menz, Rihm, & Buchel, 2016). The three measures of REM consolidation from Night 2 were correlated with responses to the CS+ early in the Recall session. None of the correlations reached statistical significance (REM latency: $r = .10$; REM efficiency: $r = -.234$, REM percent: $r = -.30$; $p > .30$ for all three correlations).

Though not connected to the main aims of this study, exploratory analyses were then conducted to examine the relationship between REM sleep consolidation on Night 3 and subsequent safety re-learning at the Recall session. A difference score was created (Block 1 – Block 2) to create a continuous variable representing the slope of safety re-learning at the Recall session on Day 3. As in the Acquisition session (see above), large numbers indicated rapid safety re-learning, as large numbers suggested participants learned to modulate their fear response in response to the CS- after relatively few trials. Inversely, comparably small numbers indicated impaired safety re-learning, as small numbers indicated participants were not able to modulate their fear response to the CS- despite multiple presentations of this cue without pairing with the aversive stimulus during this testing session as well as during the previous two testing sessions. Bivariate correlation was then used to examine the relationship between REM latency, REM efficiency, and REM percent on the previous night (Night 3) and this measure of the slope of safety re-learning at the subsequent Recall session. See Table 5 for these results. There was a significant relationship
between REM percent on Night 3 and the slope of subsequent safety re-learning ($r = .69, p = .009$). Neither REM latency ($r = .168, p = .583$) nor REM percent on Night 3 ($r = .22, p = .474$) was associated with the slope of safety re-learning.

Sections of Chapter 3 of this dissertation will be submitted for publication with co-authors, Norman, Sonya; Drummond, Sean; Risbrough, Victoria. The dissertation author was the primary investigator and will be the primary author on this publication.
CHAPTER 4. DISCUSSION

Though research in animals and healthy humans has shown critical links between REM sleep and fear and safety learning, this was the first study to investigate this relationship in patients with PTSD. This small study (n = 13), conducted in veterans with PTSD at the San Diego VA Medical Center, investigated this relationship using in-lab sleep assessment and a classical conditioning paradigm over a three-day period. In line with our hypotheses about associations between safety learning and subsequent REM sleep consolidation, participants who more rapidly learned the safety signal on the first day of testing had more efficient REM sleep that night. Subsequently, participants who had more REM percent on Night 3 more rapidly re-learned safety on the final day of testing. Contrary to our hypotheses and contrary to the extant literature in animals and healthy humans, no relationships were found between REM sleep and responsiveness to the CS+ during any of the testing sessions, including the extinction recall session. This study, conducted on a relatively young, ethnically diverse sample of veterans with moderately severe PTSD, insomnia, and depressive symptoms, adds to the literature demonstrating links between overnight sleep and daytime safety learning processes. However, this study did not replicate research in animals and healthy human control populations showing similar associations between REM sleep overnight and daytime fear extinction processes.

PTSD, Fear Extinction, and Safety Learning

Results from the psychophysiological testing sessions suggest these PTSD patients successfully underwent fear conditioning, extinction learning, and extinction recall. According to analyses of the fear conditioning and extinction learning data
alone, participants successfully underwent fear conditioning in that they learned to respond more anxiously to the CS+ than they did to the CS- after repeated pairing of the CS+ with an aversive stimulus during the fear acquisition session. Results from the extinction learning session revealed participants successfully underwent extinction, in that they responded more anxiously to the CS+ than they did to the CS- at the beginning of the extinction session, and learned over the course of the extinction learning session that the CS+ no longer signaled threat and responded similarly to both cues. At the beginning of the recall session 24 hours after extinction learning, participants responded similarly to the CS+ and CS- due to successfully learning the previous day that the CS+ was now safe. According to prior laboratory studies in PTSD populations, PTSD patients show abnormalities in fear conditioning (Grillon & Morgan, 1999; Orr et al., 2000), extinction learning (Wessa & Flor, 2007), and extinction recall (Milad et al., 2008) in comparison to other clinical populations and healthy controls. Clinically, this is related to re-experiencing symptoms of PTSD, as PTSD patients respond anxiously to cues associated with trauma, even long after the trauma. Though fear processes have long been suspected to be critical in PTSD symptoms (e.g., Rothbaum & Davis, 2003; Myers & Davis, 2007; Quirk & Mueller, 2008), recent findings demonstrate the importance of impaired safety learning as well. Deficits in safety learning are also related to daytime symptoms of PTSD, as PTSD patients have difficulty distinguishing from threatening and safe cues and environments, leading to overgeneralization of avoidance as well as hypervigilance symptoms (Jovanovic et al., 2012). Laboratory studies have demonstrated PTSD patients have difficulty learning safety signals in the context of fear conditioning studies, even when compared to patients with other clinical disorders such as anxiety.
and/or depression (Jovanovic et al., 2010, Acheson et al., 2015). It is worthy to note that participants in this study showed a large increase in potentiation to both the CS+ and CS- at the beginning of the Extinction session relative to the end of the acquisition session the prior day, and this relative increase in anxious responding may have occurred to a greater extent than would be expected compared to studies using the same paradigm in healthy human control participants (e.g., Straus et al., 2017). Without an age-matched control group in this study for comparison purposes, however, it is difficult to determine to what extent this increase in anxious responding to both cues during early Extinction was associated with PTSD diagnosis for these participants.

**Safety Learning and REM Sleep**

A growing body of research implicates sleep, and REM sleep in particular, to be critical for safety learning. A study from our research group, conducted in healthy human subjects, showed safety learning is related to REM sleep consolidation (Marshall et al., 2014), which then has implications for subsequently differentiating between threat and safety. In this study in PTSD patients, worse safety learning at the fear acquisition session on Day 1 was associated with less REM efficiency that night. This replicates findings from studies in healthy humans (Marshall et al., 2014), and underscores the importance of daytime PTSD symptoms and their relationship with subsequent sleep disruption—patients who were less able to discriminate between safe and dangerous cues showed more disrupted REM sleep. Additionally, this study showed that PTSD patients who had less consolidated REM sleep on Night 3 showed more subsequent difficulties with safety signal re-learning, which further
validates our theoretical model (see Figure 1) hypothesizing that REM sleep disturbances play a role in the maintaining daytime PTSD symptoms in the long term.

The associations between safety learning and REM sleep found in this study are understandable given the hypothesized links between sleep and the biological processes involving fear and safety learning. Fear conditioning involves activation of central stress responses, involving the limbic system (Phillips & LeDoux, 1992) as well as the noradrenergic system, which is associated with fragmentation of REM sleep (Raskind et al, 2007). In the current study, less efficient safety signal learning was associated with more fragmented REM sleep the following night, suggesting that the PTSD patients who were less able to modulate the fear response during acquisition had increased activation of the limbic system and increased production of norepinephrine, which may have then in turn resulted in fragmented REM. In addition to activation of limbic and noradrenergic pathways, studies have also shown inhibition of fear, such as safety signal learning, involves activation of frontal brain regions such as the vmPFC to serve as a “top-down” process for modulating fear response (Jovanovic & Norrholm, 2011). Studies in healthy control participants show sleep disruption results in increased activation in the amygdala as well as decreased connectivity the frontal regions needed to modulate expression of that fear response (van der Helm et al., 2011; Nieuwenhuis & Takashima, 2011). In this study, REM sleep disruption was associated with impaired ability to “re-learn” the safety signal on the last day of testing. Though we did not measure these brain systems or neurotransmitters directly in this study, the results from this study are in line with what would be expected given the hypothesized links between REM sleep and safety
learning, which provides further evidence showing sleep disruption is a critical factor in the development and maintenance of daytime PTSD symptoms.

**REM Sleep and Extinction Processes**

Contrary to our hypotheses, no relationship was found between REM sleep consolidation and reactivity to the threat signal (CS+), either during the extinction learning session or during the extinction recall session the following day. Prior studies in healthy human control participants have shown that strong safety signal learning is associated with more consolidated REM sleep, which in turn is associated with enhanced ability to discriminate between threat and safety signals during early extinction learning (Marshall et al., 2014), an effect that was not observed in this study. Additionally, many studies in animals (Fu et al., 2007; Datta & O’Malley, 2013) as well as healthy human control participants (Straus et al, 2017, Spoormaker et al, 2010, Spoormaker et al, 2012) have shown that more fragmentation of REM sleep is associated with more anxious responding to a CS+ during recall of extinguished fear. These results were also not duplicated in this study.

There are a variety of possible explanations for these null results. Notably, this study was the first to examine relationships between REM sleep and extinction processes in PTSD. All previous studies investigating these questions have been conducted in animals and/or healthy human control participants. Often, studies in animals or healthy human control subjects directly manipulate sleep by use of total sleep deprivation (Straus et al., 2017), or selective REM sleep deprivation (Spoormaker et al., 2012) and compare findings to a control group that did not undergo the sleep manipulation. These interventions may result in more easily observable effects on daytime fear conditioning and extinction parameters, and this
type of design allows for the ability to determine the causal effects of sleep disruption on fear and extinction parameters. This study did not directly manipulate REM consolidation and thus was a correlational study designed to examine naturalistic variations in REM sleep in PTSD patients, attempting to determine whether or not REM sleep consolidation was associated with fear parameters. Future studies could use a manipulation to either disrupt REM sleep (via REM deprivation) or consolidate REM sleep overnight (e.g., via administration of a pharmacological agent such as prazosin), and examine the relationship between REM sleep and extinction processes in PTSD patients. These findings may more closely replicate the research in animals and healthy humans.

Perhaps the most important consideration for these null results is the small size of this study and thus the resultant lack of statistical power to detect effects even if they are present. Notably, previous studies showing links between sleep and extinction processes in control populations have shown effect sizes in the medium range, ranging from $R^2 = .17$ (Straus et al., 2017) to $R^2 = .25$ (Marshall et al., 2014). Making the conservative assumption that effect sizes would be similar in PTSD patients, is estimated that 30 subjects would be necessary to have an 80% chance of finding a medium effect of the relationship between REM consolidation and extinction recall with an alpha set at .05 and with three predictors in the model (representing the three measures of REM sleep consolidation used here). This study enrolled far fewer participants. Notably, results from the correlations between REM sleep and fear parameters in this study were often in the expected direction, but not statistically significant. For example, more REM consolidation on Night 2 was associated with less anxious responding to the CS+ early in the extinction learning session the next
day, which would replicate previous studies showing this same relationship in healthy human control subjects. In this study, effect sizes for the association between REM sleep and extinction recall ranged up to $r = .48 \ (R^2 = .23$, within the range of effect sizes found in the studies in controls), so none of these relationships reached statistical significance given the small number of participants enrolled in this study. The question about the relationship between REM sleep and extinction processes in PTSD patients remains open; larger studies and more statistical power may be needed to detect these relationships.

**Clinical Implications**

The current study sheds light on the potential mechanistic role of sleep disturbance in the pathogenesis of PTSD. Sleep disruption is common to PTSD, with some studies suggesting sleep disruption in 70% to 91% of PTSD patients (Neylan et al., 1998). Additionally, exposure to trauma increases the risk for sleep disturbance, which often precedes development of PTSD (Babson & Feldner, 2010). In particular, REM sleep fragmentation in the early aftermath of trauma predicts the later development of PTSD symptoms (Mellman et al., 2007). Trauma exposure may result in sleep disturbances such as insomnia or nightmares, which is associated with fragmented REM sleep. This sleep disruption in turn interferes with the ability to differentiate threat from safety signals, leading to overgeneralization of avoidance and hypervigilance, making the development of PTSD more likely. According to emotional processing theory, avoidance of feared cues in particular has been hypothesized to be a main mechanism by which PTSD symptoms are perpetuated in the long term. As discussed in reviews of the extant literature on sleep and PTSD (Germain, Buysse, & Nofzinger, 2008; Germain, 2013; Spoormaker & Montgomery, 2008), sleep
disturbance, and REM fragmentation in particular, may activate central stress system processes that interfere with safety learning and promote PTSD symptoms. In addition, these results suggest that initiating exposure therapy in the context of ongoing sleep disruption may render treatment less effective due to sleep disruption–related impairments in the ability to differentiate threat from safety. Furthermore, as REM sleep disruption does not appear to affect within-session extinction learning, it may appear to the therapist as though the exposure therapy is effective when anxiety to a feared cue decreases during a given exposure activity. However, in patients with poor sleep, impairments in safety learning are more likely to resurface at a later time, potentially increasing hyperarousal and anxious avoidance of feared cues despite successful extinction learning. This study adds to the literature suggesting that it is important to attend to sleep disruption when deciding to initiate exposure therapy and throughout the therapy process itself. This study further suggests that normalizing sleep disruption via behavioral or pharmacological methods before initiation of exposure therapy should facilitate optimal response to trauma-focused interventions.

Future research should evaluate these hypotheses directly (see below).

Limitations and Future Directions

Though this study established important links between REM sleep disturbance and safety learning processes in PTSD patients, there are a few limitations worth noting. First, this was a small exploratory study, which resulted in limited power to examine relationships between sleep and daytime fear learning. This study did not find hypothesized links between REM sleep and extinction processes. As noted above, the small size of this study results in a lack of statistical power to detect effects even if they are present. Additionally, the small sample size of this study means that
the results may not be generalizable, and the current results may lack stability. Future research should scale up and re-examine potential relationships between REM sleep and fear/extinction parameters. Second, although this study provides more evidence for potential links between fear responses, brain networks, neurotransmitters, and sleep disruption, this study only examined behavioral responses. Future studies should include neuroimaging or other biological sampling to examine hypothesized links between fear learning, neural networks, and neurotransmitters. Third, due to recruitment concerns, we did not carefully control for psychotropic use that may have affected sleep continuity during the in-lab portion of this study. Future studies could limit participation to medication-free participants, or control for medication use for a more “pure” test of study hypotheses.

Despite these limitations, this study extends previous research and adds to the literature suggesting sleep disturbance interferes with safety learning, indicating that sleep disruption is an important factor in the development and maintenance of daytime PTSD symptoms. This study also provides additional evidence in support of the larger hypothesized model explicating relationships between fear and safety processes, the limbic and adrenergic systems, sleep disruption, and the subsequent perpetuation of PTSD symptoms (see Germain, 2013, for review). As noted above, future research should expand the scope of this study to include functional neuroimaging, which would provide additional evidence validating the model. In addition, this study also has implications for future research involving PTSD treatment. Sleep disruption is associated with fear and safety learning processes, both of which are critical for response to exposure-based interventions such as Prolonged Exposure. Future research should examine fear, safety, and sleep
parameters as part of an intervention study, which will allow researchers to directly test the hypotheses that disrupted sleep interferes with exposure-based treatment. Additionally, future research should examine whether treating sleep disturbance, via medication-based or behavioral treatments, enhances response to evidence-based PTSD interventions. In sum, sleep disruption appears to be a modifiable process to target in PTSD intervention.

Sections of Chapter 4 of this dissertation will be submitted for publication with co-authors, Norman, Sonya; Drummond, Sean; Risbrough, Victoria. The dissertation author was the primary investigator and will be the primary author on this publication.
Table 1. Demographics of the study sample

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>31.88</td>
<td>7.67</td>
</tr>
<tr>
<td>Male</td>
<td>11</td>
<td>84.6</td>
</tr>
<tr>
<td>Female</td>
<td>2</td>
<td>15.4</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>American Indian or Alaskan Native</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Asian</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Black or African American</td>
<td>1</td>
<td>7.7</td>
</tr>
<tr>
<td>Caucasian</td>
<td>11</td>
<td>84.6</td>
</tr>
<tr>
<td>Native Hawaiian or Other Pacific Islander</td>
<td>1</td>
<td>7.7</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>5</td>
<td>38.5</td>
</tr>
<tr>
<td>Non-Hispanic</td>
<td>8</td>
<td>61.5</td>
</tr>
<tr>
<td>Military Branch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Army</td>
<td>4</td>
<td>30.8</td>
</tr>
<tr>
<td>Navy</td>
<td>3</td>
<td>23.1</td>
</tr>
<tr>
<td>Marine Corps</td>
<td>6</td>
<td>46.2</td>
</tr>
<tr>
<td>Air Force</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS Grad</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Some College</td>
<td>11</td>
<td>84.6</td>
</tr>
<tr>
<td>Completed Bachelor's Degree</td>
<td>1</td>
<td>7.7</td>
</tr>
<tr>
<td>Completed Master’s Degree</td>
<td>1</td>
<td>7.7</td>
</tr>
</tbody>
</table>
Table 2. Clinical characteristics of the study sample

<table>
<thead>
<tr>
<th>Criterion A Trauma Type</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combat</td>
<td>10</td>
<td>76.9</td>
</tr>
<tr>
<td>Military Sexual Trauma</td>
<td>2</td>
<td>15.4</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>7.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAPS-5 Score</td>
<td>42.85</td>
<td>12.22</td>
</tr>
<tr>
<td>PCL-S Score</td>
<td>55.78</td>
<td>16.29</td>
</tr>
<tr>
<td>PHQ-9 Score</td>
<td>14.31</td>
<td>6.94</td>
</tr>
</tbody>
</table>
Table 3. Sleep summary data

<table>
<thead>
<tr>
<th>Questionnaires</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Insomnia Severity Index</strong></td>
<td>14.77</td>
<td>5.63</td>
</tr>
<tr>
<td><strong>PSQI Global Score</strong></td>
<td>11.86</td>
<td>3.74</td>
</tr>
<tr>
<td><strong>PSQI Addendum for PTSD</strong></td>
<td>10.77</td>
<td>4.59</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sleep Diaries (baseline week)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time in Bed (minutes)</strong></td>
<td>472.98</td>
<td>64.11</td>
</tr>
<tr>
<td><strong>Total Sleep Time (minutes)</strong></td>
<td>352.85</td>
<td>80.81</td>
</tr>
<tr>
<td><strong>Sleep Latency (minutes)</strong></td>
<td>42.06</td>
<td>25.47</td>
</tr>
<tr>
<td><strong>Wake After Sleep Onset (minutes)</strong></td>
<td>74.31</td>
<td>54.27</td>
</tr>
<tr>
<td><strong>Sleep Efficiency (%)</strong></td>
<td>74.59</td>
<td>12.40</td>
</tr>
<tr>
<td><strong>Total Number of Nightmares</strong></td>
<td>7.00</td>
<td>7.02</td>
</tr>
<tr>
<td><strong>Average Nightmare Intensity</strong></td>
<td>5.29</td>
<td>3.83</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Actigraphy (baseline week)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Sleep Time (minutes)</strong></td>
<td>366.64</td>
<td>43.83</td>
</tr>
<tr>
<td><strong>Wake After Sleep Onset (minutes)</strong></td>
<td>45.13</td>
<td>17.56</td>
</tr>
<tr>
<td><strong>Sleep Efficiency (%)</strong></td>
<td>76.98</td>
<td>7.46</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Polysomnography (average over 3 nights)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Sleep Time (minutes)</strong></td>
<td>388.03</td>
<td>54.27</td>
</tr>
<tr>
<td><strong>Sleep Latency (minutes)</strong></td>
<td>17.90</td>
<td>18.52</td>
</tr>
<tr>
<td><strong>Wake After Sleep Onset (minutes)</strong></td>
<td>28.51</td>
<td>26.56</td>
</tr>
<tr>
<td><strong>Sleep Efficiency (%)</strong></td>
<td>89.85</td>
<td>6.20</td>
</tr>
</tbody>
</table>
Table 4. Safety Learning and Subsequent REM Sleep Consolidation

<table>
<thead>
<tr>
<th>Variables</th>
<th>REM Latency</th>
<th>REM Efficiency</th>
<th>REM Percent</th>
<th>Safety Learning Slope</th>
<th>Safety Learning Magnitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rem Latency</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REM Efficiency</td>
<td>-.170</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REM Percent</td>
<td>-.332</td>
<td>.489</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Safety Learning Slope</td>
<td>.168</td>
<td>.607*</td>
<td>.218</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Safety Learning Magnitude</td>
<td>.075</td>
<td>.282</td>
<td>.074</td>
<td>.489</td>
<td>--</td>
</tr>
</tbody>
</table>

* $p < .05$
Table 5. REM Sleep Consolidation and Subsequent Fear and Safety Recall

<table>
<thead>
<tr>
<th>Variables</th>
<th>REM Latency</th>
<th>REM Efficiency</th>
<th>REM Percent</th>
<th>Extinction Recall</th>
<th>Safety Re-Learning Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rem Latency</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REM Efficiency</td>
<td>-.514</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REM Percent</td>
<td>.093</td>
<td>.533</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extinction Recall</td>
<td>.380</td>
<td>-.187</td>
<td>.166</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Safety Re-Learning Slope</td>
<td>.069</td>
<td>.315</td>
<td>.688**</td>
<td>-.154</td>
<td>--</td>
</tr>
</tbody>
</table>

** p < .01
Figure 1. Explanatory Model
Figure 2. Timeline of Testing Phase
Figure 3. Summary of Recruitment and Enrollment

100 Veterans Contacted

- 56 Veterans did not respond to initial attempt to contact

44 Telephone Screens Conducted

- 18 Veterans ineligible by telephone screen

26 Veterans attended in-person eligibility appointment

- 6 Veterans ineligible
- 5 Veterans declined

15 Veterans began testing phase of study

- 1 Veteran dropped after Night 1
- 1 Veteran withdrew after Night 2

13 Veterans completed entire study
Figure 4. Fear Potentiated Startle Testing Procedures. Day 1 of testing (Fear/Safety Learning) included 8 presentations of the CS+ (blue circle), paired with an air puff to the throat 75% of the time. Day 1 also included 8 presentations of the CS- without being paired with the air puff, and 8 Noise Alone (NA) trials. Day 2 (Extinction Learning) included 16 presentations of the CS+, 16 presentations of the CS-, and 16 NA trials. No air puffs were administered during this session. Day 3 (Extinction Recall) included 8 CS+, 8 CS-, and 8 NA trials. As on the day before, no air puffs were administered.
Figure 5. Fear Acquisition Session
Figure 6. Extinction Learning Session
Figure 7. Extinction Recall Session
REFERENCES


MC USNR, R. N. M., & Volkert, S. L. (2010). Insomnia is the most commonly reported symptom and predicts other symptoms of post-traumatic stress disorder in US service members returning from military deployments. Military Medicine, 175(10), 759.


