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ORIGINAL ARTICLE

The relationship of fear-potentiated startle and polysomnography-measured sleep in trauma-exposed men and women with and without PTSD: testing REM sleep effects and exploring the roles of an integrative measure of sleep, PTSD symptoms, and biological sex

Anne Richards[1,2,*], Sabra S. Inslicht[1,2], Leslie M. Yack[2], Thomas J. Metzler[2], J. Russell Huie[1,2], Laura D. Straus[1,2], Cassandra Dukes[2], Samantha Q. Hubachek[2], Kim L. Felmingham[3], Daniel H. Mathalon[1,2], Steven H. Woodward[4] and Thomas C. Neylan[1,2]

[1]Department of Psychiatry and Behavioral Sciences, University of California, San Francisco, San Francisco, CA, USA, [2]San Francisco VA Medical Center (SFVAMC), San Francisco, CA, USA, [3]University of Melbourne, Parkville, Victoria, Australia and [4]National Center for PTSD and VA Palo Alto Health Care System, Palo Alto, CA, USA

*Corresponding author. Anne Richards, San Francisco VA Medical Center, 4150 Clement Street, San Francisco, CA 94121, USA. Email: anne.richards@ucsf.edu.

Abstract

Study Objectives: Published research indicates that sleep is involved in emotional information processing. Using a fear-potentiated startle (FPS) and nap sleep protocol, we examined the relationship of emotional learning with REM sleep (REMS) in trauma-exposed participants. We also explored the roles of posttraumatic stress disorder (PTSD) symptoms, biological sex, and an integrative measure of polysomnography-measured (PSG) sleep in the learning-sleep relationship.

Methods: After an adaptation nap, participants ($N = 46$) completed two more visits (counterbalanced): a stress-condition visit, which included FPS conditioning procedures prior to a nap and assessment of learning retention and fear extinction training after the nap, and a control visit, which included a nap opportunity without stressful procedures. FPS conditioning included a "fear" visual stimulus paired with an air blast to the neck and a "safety" visual stimulus never paired with an air blast. Retention and extinction involved presentation of the visual stimuli without the air blast. Primary analyses examined the relationship between FPS responses pre- and post-sleep with stress-condition REMS duration, controlling for control-nap REMS duration.

Results: Higher safety learning predicted increased REMS and increased REMS predicted more rapid extinction learning. Similar relationships were observed with an integrative PSG sleep measure. They also showed unexpected effects of PTSD symptoms on learning and showed biological sex effects on learning-sleep relationships.

Conclusions: Findings support evidence of a relationship between adaptive emotional learning and REMS. They underscore the importance of examining sex effects in sleep-learning relationships. They introduce an integrative PSG sleep measure with potential relevance to studies of sleep and subjective and biological outcomes.

Statement of Significance

Sleep disturbance is a core feature of posttraumatic stress disorder (PTSD) and contributes to the development and maintenance of the disorder, however, mechanisms linking stress and sleep are still poorly understood. Early research suggests that impairments in safety signal learning are associated with disruption of REM sleep, a sleep stage that is considered critical for sleep-dependent emotion information processing. Using a nap protocol in trauma-exposed subjects with varying PTSD severity, our findings strengthen preliminary evidence demonstrating a relationship between sleep, safety signal learning and extinction learning, underscore the importance of examining sex differences, and suggest a novel approach for measuring the relationship of polysomnography sleep with psychological and neurobiological outcomes.

Key words: fear conditioning; sleep; REM sleep; PTSD; polysomnography; fear-potentiated startle

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Introduction

Sleep disturbance is a core feature of posttraumatic stress disorder (PTSD) [1–4] and abnormalities in sleep contribute to the development and maintenance of the disorder [5]. One model linking sleep disturbance and PTSD posits that anxious emotions and cognitions, such as those resulting from a traumatic exposure, negatively impact sleep quality. The resulting disruptions in sleep then contribute to defective emotion regulation and stress-related information processing, contributing to a positive feedback loop of daytime stress symptoms and nighttime disturbance [6, 7]. However, existing models remain largely theoretical: the study of the biological mechanisms underlying the link between sleep and stress symptoms is in its infancy.

Central to the dominant models of PTSD is also the concept that exposure to a traumatic stressor engages an associative emotional learning process, wherein individuals learn to associate threat-specific stimuli from the trauma context with fear and heightened physiological stress responses, and, if responding adaptively, learn to associate nonthreatening stimuli from the trauma context with safety feelings and dampened physiological responses (for reviews, see Refs. [8, 9]). Following trauma exposure, extinction processes normally lead to the gradual reduction of learned (i.e. conditioned) responses when conditioned stimuli are repeatedly presented in the absence of actually threatening content. Despite some inconsistencies, overall the research indicates that PTSD is associated with excessive fear learning and deficits in adaptive extinction processes [8]. Recent research also indicates that defects in safety signal learning, or the ability to learn that nonthreatening stimuli in the trauma context are safe, and to discriminate between threat and safety stimuli *after* the traumatic exposure (aka "differential" conditioning), may be a central feature of maladaptive fear learning in PTSD [8–10].

Linking fear and safety learning to sleep, two recent studies of overnight sleep reported links between REM sleep (REMS) and adaptive learning. In a study of healthy participants, Marshall *et al.* found that greater safety signal learning was associated with a composite measure of post-learning REMS, involving REMS duration, REMS latency, and REMS percent [11]. They also found that this measure of REMS was associated with higher retention of safety signal learning and differential conditioning after sleep. Similarly, in a pilot study of 18 male military veterans with PTSD, Straus *et al.* found that more rapid safety signal learning was associated with greater REMS efficiency (epochs scored as REMS within a period of REMS) in post-learning sleep [12]. They also found that higher REMS percent (percent of all sleep epochs scored as REMS) was associated with a higher value on a measure of safety signal recall in an extinction recall session following initial extinction.

While these studies provide early, compelling evidence of a link between fear and safety signal learning and REMS, replication in larger samples is critical to solidify our confidence in the importance of REMS in adaptive learning processes [13], and to compare and contrast these relationships in trauma-exposed individuals with and without the PTSD symptoms that fear and safety learning are expected to model. In fact, a newly published meta-analysis by Schenker *et al.* which examines the relationship between sleep and both fear and safety signal responses during fear conditioning and extinction underscores the lack of consistency in findings across studies, and the importance of examining sex differences in these relationships [14]. Notwithstanding some acknowledged limitations in Schenker *et al.*'s methods, including their approach to measuring fear and safety learning as an average of physiological responses rather than a change in response within testing sessions, their findings are noteworthy with respect to REMS effects: the relationship of fear conditioning to subsequent REMS percent and of REMS percent to subsequent extinction went in the opposite direction for male versus female subjects.

Additionally, REMS is nested within a cycle of multiple sleep stages, typified by an initial progression from wake to light (N1) to deep (N3) sleep followed by a transition to REMS, followed by a repeated cycling between NREMS and REMS. Published research increasingly highlights the critical importance of NREMS stages for emotional recalibration (i.e. N3: [14,15]), and information processing and emotional memory (i.e. N2: [16]). The above-mentioned meta-analysis also found that reduced slow wave (N3) and increased N2 sleep percentages in sleep after fear conditioning (prior to extinction learning) were associated with higher physiological responses to the fear and/or safety signal during extinction learning [14]. Their analyses also indicated that the effects of N2 and N1 differed in clinical (i.e. insomnia disorder and PTSD) versus healthy control subjects. While the role of N2 sleep in PTSD has not been fleshed out, N3 is convincingly deficient and N1 and wake after sleep onset (WASO) increased in PTSD relative to healthy controls [17–20]. Preliminary work by Kleim *et al.* is also suggestive that characteristics of N2 sleep play a role in adaptive processing of laboratory trauma [16]. Together, these findings suggest that exploration of NREMS relationships with fear learning should be performed. Finally, these findings suggest that examining sleep as an integrated whole of interdependent parts is indicated. The potential contributions of various stages of sleep may be dependent on the quantity of other stages of sleep as well as sleep disruption by WASO, such that examination of each stage in isolation of other features of the sleep period may miss important information about an integrated sleep influence on adaptive fear learning, or may be misleading. For example, it is not clear whether the opposite-direction effects of N3 and N2 percentages observed by Schenker *et al.* are attributable to independent effects of N3 and N2, or whether one may be a spurious result due to tradeoffs between N3 and N2 components. Specialized data analytic methods, called compositional data analysis, have been developed to work with data in which components are constrained to sum to a constant whole [21, 22]. To examine the influence of an integrative measure of sleep period components, these compositional data analytic methods were used (see Methods section for details).

Altogether, the published findings indicate that (1) replication of findings that REMS plays an adaptive function in fear learning in larger samples is sorely needed; (2) the role of sleep stages other than REMS and the role of a sleep as an integrated whole needs to be examined in relationship to fear learning; and (3) examination of sex effects and PTSD symptom effects must be incorporated into this research. To contribute to addressing these gaps in the published research, this study examines the effects of fear and safety learning on subsequent sleep, and the effect of subsequent sleep on retention and extinction of fear and safety learning, using a nap protocol, in trauma-exposed men and women with varying PTSD severity. Utilizing a nap protocol with a stress and a

control nap condition, we hypothesized that greater safety learning (stress condition) would predict a greater increase in subsequent REMS duration, controlling for REMS duration in the control condition (Hypothesis 1). Next, we predicted that greater REMS duration (stress condition) would predict greater retention of safety signal learning (Hypothesis 2a) and differential conditioning (Hypothesis 2b) and more rapid extinction of conditioned responses (Hypothesis 2c), controlling for REMS duration in the control condition. Next, based on an integrative measure of the sleep period derived from factor analysis, we examined whether sleep richer in REMS and N3 relative to N1 and WASO behaved similarly to REMS with respect to adaptive learning. We hypothesized that higher fear potentiation (Hypothesis 3a) and lower safety signal learning (Hypothesis 3b) (stress condition) would be associated with a reduced balance of REM/N3 relative to N1/WASO, controlling for this balance in the control condition nap. We also hypothesized that a greater balance of REM/N3 relative to N1/WASO in the stress condition nap would predict greater retention of safety learning (Hypothesis 4a) and differential conditioning (Hypothesis 4b) and more rapid extinction of the fear response (Hypothesis 4c), controlling for this balance in the control condition nap. Fifth, we examined the relationship between PTSD symptoms and fear-potentiated startle (FPS), and predicted that higher CAPS-measured PTSD symptoms would be associated with greater fear potentiation (Hypothesis 5a), reduced safety signal learning (Hypothesis 5b), and poorer extinction of conditioned responses (5c). We performed exploratory and post-hoc analyses to further examine the relationship of standard polysomnography (PSG) variables with fear learning variables and to examine the role of PTSD symptoms and biological sex in the relationships between fear and safety learning and sleep. See Table 1 for a consolidated list of study hypotheses for easy reference.

Methods

Participants

Forty-six male (n = 24) and female (n = 22) participants with a history of criterion A trauma exposure and aged 18–50 were recruited at the San Francisco VA Medical Center as part of a study of sleep, emotional memory, and PTSD. After written informed consent procedures, participants underwent screening assessments, a medical evaluation, and a clinical interview including the clinician-administered PTSD scale (CAPS) [23] and the structured clinical interview for DSM-5 (SCID-5) [24] to ascertain presence or absence and symptom levels of current and past PTSD and to diagnose other current and past psychiatric disorders. Participants with a lifetime history of bipolar disorder or a psychiatric disorder with psychotic features were excluded, as were individuals with a severe substance use disorder in the prior year, recent history of consumption of >14 standard drinks per week, or positive urine drug screen at any visit. Pregnancy or evidence of peri- or post-menopausal status was an exclusion criterion. Participants with medical diagnoses or medications significantly impacting sleep or cognitive function, or prescribed standing bedtime medications targeting sleep, were excluded. Participants were evaluated for sleep apnea at home using an ApneaLink device (ResMed, San Diego, CA), and participants with obstructive sleep apnea (OSA) were required to complete naps using a continuous positive airway pressure (CPAP) device. Untreated OSA (defined by an apnea-hypopnea index [AHI] > 10) was an exclusion. Eligible participants were subsequently scheduled for three nap visits. Participants were asked to maintain a regular sleep schedule during the 7 days preceding a nap visit, defined as a bedtime between 10 pm and 12 am and a wake time between 6 am and 8 am. They were asked to limit caffeine to one cup in the morning on the day of nap visits and to limit alcohol consumption, for which adherence was monitored via sleep diary.

Table 1. Study hypotheses

Hypothesis 1	Greater safety learning (stress condition) predicts a greater increase in REMS duration (stress condition nap), controlling for REMS duration in the control condition nap.
Hypothesis 2a	Greater REMS duration (stress condition nap) predicts greater retention of safety signal learning, controlling for REMS duration in the control condition nap.
Hypothesis 2b	Greater REMS duration (stress condition nap) predicts greater retention of differential conditioning, controlling for REMS duration in the control condition nap.
Hypothesis 2c	Greater REMS duration (stress condition nap) predicts more rapid extinction of conditioned responses, controlling for REMS duration in the control condition nap.
Hypothesis 3a	Higher fear potentiation predicts a reduced balance of REM and N3 relative to N1 and WASO (stress condition nap) as reflected in an integrated measure of sleep derived from factor analysis, controlling for this balance in the control condition nap.
Hypothesis 3b	Lower safety signal learning predicts a reduced balance of REM and N3 relative to N1 and WASO (stress condition nap) as reflected in an integrated measure of sleep derived from factor analysis, controlling for this balance in the control condition nap.
Hypothesis 4a	A higher balance of REM and N3 relative to N1 and WASO (stress condition nap) predicts greater retention of safety learning, controlling for this balance in the control condition nap.
Hypothesis 4b	A higher balance of REM and N3 relative to N1 and WASO (stress condition nap) predicts greater retention of differential conditioning, controlling for this balance in the control condition nap.
Hypothesis 4c	A higher balance of REM and N3 relative to N1 and WASO (stress condition nap) predicts more rapid extinction of the conditioned responses, controlling for this balance in the control condition nap.
Hypotheses 5a	Higher CAPS-measured PTSD symptoms predict greater fear potentiation.
Hypothesis 5b	Higher CAPS-measured PTSD symptoms predict reduced safety signal learning.
Hypothesis 5c	Higher CAPS-measured PTSD symptoms predict poorer extinction of conditioned responses.
Exploratory analyses	We performed exploratory and post-hoc analyses to further examine the relationship of standard PSG variables with fear learning variables and to examine the role of PTSD symptoms and biological sex in the relationship between fear and safety learning and sleep.

Overview of nap visits

Participants attended three nap visits, each separated by at least 6 to 7 days to reduce the likelihood that prior naps would impact later naps via sleep/wake rhythm disruption (Figure 1A). The first nap visit consisted of an adaptation nap, during which participants acclimated to the experience of the PSG hook-up and PSG-monitored sleep in the sleep laboratory. Participants arrived at approximately 11:00 am, were provided with a meal, were prepared for the PSG study, and started the nap opportunity with lights-out at 13:30. The second and third naps were the control nap, during which minimal and non-stressful procedures were carried out prior to the nap, and a stress condition nap, which included FPS procedures in the context of an activity-packed morning prior to the nap (Figure 1B). For both the control and stress visits, participants arrived at the lab by 9:00 am, at which time they performed a urine drug screen to rule out street drug and benzodiazepine use. In the control condition nap, morning activities consisted of completion of brief self-report surveys (9:00, 10:30), an approximately 40-minute cognitive battery without explicit emotional content (9:45), and unstructured activities such as reading, or emailing or talking on cell phones until lunch. Participants were allowed to take a brief walk (15–30 min) on the medical center campus but were not allowed to engage in strenuous exercise, eat additional foods or drink caffeinated beverages. In the stress condition visit, participants completed self-report surveys (9:15), and set-up and electrode application for the FPS protocol (9:30). Participants were done with startle procedures between 10:15 and 10:30. Participants completed self-report surveys (10:30) and an additional laboratory task involving viewing of negative and neutral imagery (11:00–11:30). A light snack was offered around 10:45, and lunch was provided at 11:30 in both visits. PSG hook-up was performed between 12:15 and 13:30 and the nap opportunity was scheduled between 13:30 and 15:30 precisely for all visits. In the control condition, participants completed brief surveys (15:45) and were dismissed after the nap. In the stress condition, participants completed brief surveys (15:45); a psychomotor vigilance task (16:00), an image recall session (16:10), and the FPS extinction set-up and protocol starting at 16:40. All participants completed a brief nap sleep quality survey after each nap, in which they rated their nap sleep quality as "good," "fair," or "poor." Efforts were made to balance the order (second vs. third) of the control and stress condition visits to control for order effects, however, strict adherence to counterbalancing was limited to accommodate

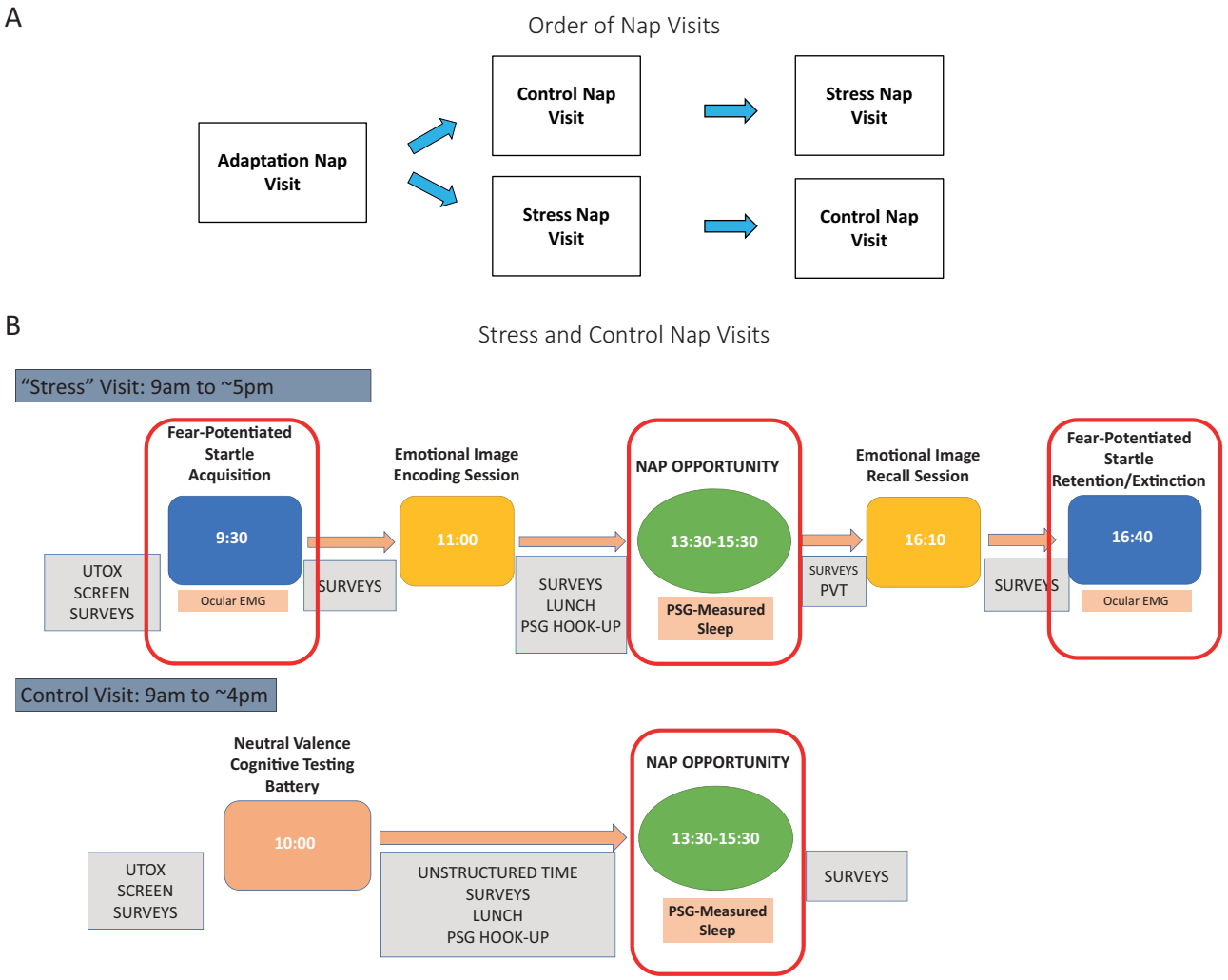


Figure 1. (A) Overall study design. All participants (N = 46) completed an adaptation visit first, followed by either the control nap visit or the stress nap visit in a counterbalanced manner. All nap visits were separated by at least 6 days. (B) Stress and control nap visit procedures. (B) depicts the full set of procedures during the control and stress visits. Fear-potentiated startle (FPS) and PSG-monitored nap opportunities contributing data to the analyses are framed in red.

participants' availability for longer (stress condition) or shorter (control condition) visits within a span of several weeks. As a result, 26 participants completed the stress visit first and 20 participants completed the control visit first. Analysis of order indicated no relationship between order of visits and sleep or fear learning variables, and therefore was not included in further analyses.

Nap sleep PSG measurement

Participants were given a 120-minute opportunity to nap under three separate conditions: adaptation, control, and stress. Daytime PSG data were visually monitored and collected at 400 Hz using the TREA Ambulatory EEG system (Natus Neurology, Pleasanton, CA) according to current American Academy of Sleep Medicine standards [25]. The standard daytime montage consisted of six standard 10–20 electroencephalography (EEG) electrode sites (F3, F4, C3, C4, O1, and O2), left and right electrooculography (EOG), three bipolar chin electromyogram (EMG), and two bipolar electrocardiograms. The EEG and EOG electrodes were referenced to contralateral electrodes (M1 and M2). The data was exported in a referential montage to EDF format to use for further analysis. All data were visually scored offline using the PRANA Production Suite software version 10 (PhiTools, Strasbourg, France). PSG data were re-referenced using a linked reference montage, filtered at .3–35 Hz, and scored in 30-second epochs according to standard AASM criteria as Wake, N1, N2, N3, or REM. Scoring was performed by two trained staff members and a final review of all PSG files was completed by one of the authors, a highly experienced registered PSG technician (L.M.Y.). Standard PSG sleep variables include sleep onset latency (SOL), REM latency (RL), time spent in each stage of sleep (N1, N1, N3, REM), total sleep time (TST), WASO, and sleep maintenance (SM). Time in bed was defined as the duration of the nap opportunity, which was 120 min in the vast majority of the cases, with the exception of a few adaptation naps during which technical challenges delayed onset of the nap opportunity. Identification of participant-specific technical challenges (e.g. extremely thick hair or beards) at the adaptation visit eliminated delays in subsequent control and stress nap opportunities. We defined SOL as the time from nap opportunity onset to the first epoch scored as any stage of sleep, RL as the time from first epoch of sleep to first epoch of REM, WASO as the time awake from sleep onset to the final epoch of wake, TST as total duration of all epochs scored as sleep (N1 + N2 + N3 + REM), and SM as the percent of the sleep period from sleep onset to final awakening that was comprised of sleep, as opposed to wake, epochs (N1 + N2 + N3 + REM/TST + WASO).

FPS procedures (stress condition only)

The FPS procedure largely resembled that described by Marshall *et al.* [11], with the exception that the unconditioned stimulus consisted of an air puff to the laryngeal prominence (Adam's apple) on the neck rather than an electric shock set at an intensity chosen by each participant (Figure 2). This aversive stimulus is a commonly utilized alternative to the electric shock that is also effective at augmenting the acoustic startle response [26]. During the acquisition (presleep) protocol, participants were first presented with a series of six noise-alone (NA) startle pulses (habituation blocks), followed by four blocks of pseudo-randomly arranged NA trials, fear-signal trials (CS+, blue circles), and safety-signal (CS–, yellow circles) trials. Each block contained two NA, two CS+, and two CS– trials. The extinction protocol consisted of eight blocks of pseudo-randomly arranged NA, CS+, and CS– trials. The sequence of trials, duration of intertrial intervals, as well as the pairing of the aversive stimulus with CS+ at .75 contingency during acquisition, was an exact replication of the sequence used by Marshall *et al.* [11]. The startle sound consisted of a 108 db, 40 ms burst of broadband noise with near-instantaneous rise and fall time delivered by headphones and a Dell laptop computer set on a table in front of the seated participant. Superlab presentation software (Cedrus Corporation, San Pedro, CA) was used to present visual stimuli on the computer monitor. The air puff, a 110-psi burst of air delivered to the participant's neck via a flexible plastic tube, was controlled by a solenoid system and triggered by Superlab. A linked Biopac MP150 system (Biopac, Inc., Goleta, CA) acquired the stimulus information and EMG data. The EMG responses to the startle sound were recorded from two Ag/AgCl electrodes placed on the left orbicularis oculi muscle, approximately 1 cm below the pupil and 1 cm below the lateral canthus, referenced to the ipsilateral mastoid bone. All electrode resistances were <10 kOhm. EMG data were recorded at a sampling rate of 1000 Hz, amplified (gain of 2000) and bandpass filtered (5–500

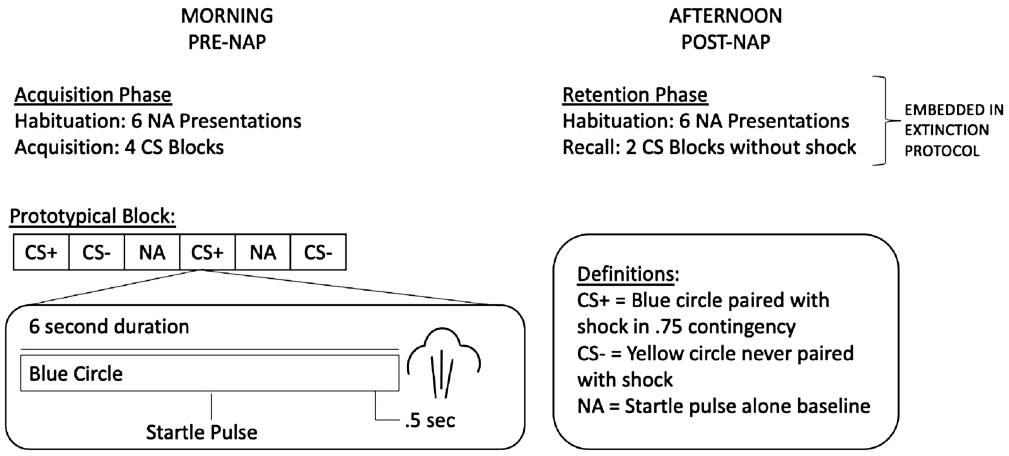


Figure 2. Fear-potentiated startle (FPS) procedures; adapted from Marshall *et al.* [11]. Schematic diagram of FPS procedures, depicting the pre-nap acquisition (aka fear conditioning) session and the post-nap retention and extinction session, as well as a prototypical block and a CS+ trial during acquisition.

Hz) as per MP150 hardware specifications. The Biopac system's Acknowledge 4.4 software recorded and stored stimulus and raw and integrated EMG response data.

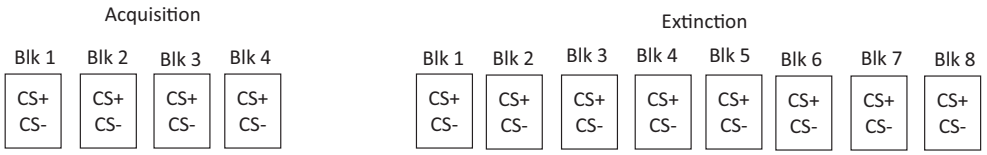
FPS data processing

MindWare software (MindWare Technologies, Inc., Gahanna, OH) was utilized for subsequent processing of EMG data and identification of peak EMG responses within the windows of interest. The peak EMG response within the 20–100 ms timeframe after the startle stimulus was utilized as the measure of startle reactivity. All windows were visually examined for artifact and cleaned prior to inclusion. The average voltage of the 20 ms window right after stimulus onset, when it is too early for a physiological response to an auditory stimulus, was subtracted from all peak responses to control for baseline noise [27]. The average baseline within participant and session (acquisition or extinction) was imputed into 20 ms windows where there was evidence of an involuntary eyeblink that would distort the baseline average. Data from all subjects were included, even if peak responses were small, to include individuals with a range of responses [28]. After the baseline was subtracted from the raw reactivity from each response, average response for each stimulus type (NA, CS+, CS–) within each block was calculated to get a single response value per stimulus type within each block. Given the inherent variation in reactivity between subjects, all values were then standardized by within-subjects response levels to facilitate comparison of differences in response between CS+, CS–, and NA across subjects [29]. The mean and standard deviation of responses to all NA trials during acquisition were calculated separately for each subject. Responses were standardized by subtracting the subject-specific NA mean from the NA, CS+, and CS– responses in each block, and dividing by the subject-specific NA standard deviation. Finally, the standardized response scores used for analysis were calculated by subtracting the standardized NA score from the standardized CS+ and CS– scores for each block. Ten standardized scores (out of 352) were identified as distributional outliers and were Winsorized at 4.0 (i.e. values greater than 4.0 were replaced with 4.0), to ensure that results were not disproportionately influenced by participants with extreme responses [30–33]. Similarly, scores from the extinction session were standardized using responses from NA trials within the extinction session only. For extinction, only one score (out of 688) was identified as an outlier, and it was deemed a recording error (17 s.d. above the mean) and was discarded.

Sleep and fear-learning variable definitions

REMS variable. REMS duration was defined by minutes spent in epochs scored as REMS.

Fear learning variables. See Figure 3 for a schematic depicting the fear learning variables. To test the hypotheses that presleep learning (acquisition) predicts sleep, two variables were calculated: fear potentiation, defined here as the CS+ signal averaged across blocks of acquisition; and safety signal learning, defined as per Marshall *et al.* [11] as the change in CS– signal from block 1 of acquisition to block 4 of acquisition, with a decrease in response indicating positive safety learning [11]. Differential conditioning, defined here as the difference in CS+ signal relative to CS– at block 4 of acquisition, was calculated to test Hypotheses 2b and 4b (sleep predicts retention of differential conditioning). We defined our fear potentiation measure utilizing data from all acquisition blocks, rather than the last blocks as done by Marshall *et al.* [11] because we observed that our participants as a whole conditioned as of block one, and that the difference between stimulus responses was stable across acquisition (see Results section and Figure 4B). Retention variables (for Hypotheses 2a–b) were defined as follows: retention of safety signal learning was defined as the standardized magnitude of the CS– during extinction block 1, adjusted for the CS– response during acquisition block 4. Greater safety signal learning retention was defined as a *lower* adjusted CS– response in extinction block 1. Retention of differential conditioning was also defined as difference between CS+ and CS– in block 1 of extinction, adjusted for this difference in block 4 of acquisition. Greater retention was defined as a greater difference in the (adjusted) CS+ versus CS– response. Extinction was defined as the



Definition of Learning Variables:

Safety Signal Learning = (CS- Acquisition Block 1) - (CS- Acquisition Block 4) (higher values indicate a decrease in the CS- response over acquisition and therefore greater safety signal learning)

Fear Potentiation = Mean CS+ in Acquisition Blocks 1-4

Differential Conditioning = (CS+ Acquisition Block 4) – (CS- Acquisition Block 4)

Safety Signal Retention = CS- in Extinction Block 1 (controlling for CS- in Acquisition Block 4; lower values indicate a smaller EMG response to the safety signal and therefore higher safety signal retention)

Differential Conditioning Retention = (CS+ Extinction Block 1) – (CS- Extinction Block 1) (controlling for Differential Conditioning in Acquisition Block 4; higher values indicate higher retention of differential conditioning)

Conditioned Response During Extinction = Mean of CS+ and CS-, in each Extinction Block (Blocks 1 – 8)

Extinction Rate = Linear Slope of Conditioned Response over Blocks 1 - 8

Figure 3. Schematic diagram of CS+ and CS– trial blocks with definitions of fear learning measures utilized in the analyses. In definitions, "CS+" and "CS–" refer to standardized scores for threat and safety signal responses respectively.

rate of decline over blocks of the mean conditioned response magnitude, including both CS+ and CS–. We tested effects of sleep variables and CAPS score on extinction via the interaction term for block by sleep or CAPS in linear mixed effects models (Hypotheses 2c, 4c, and 5c). We calculated extinction slopes for each subject for use in correlational analyses.

Integrative PSG sleep variable. We conducted a factor analysis on five EEG-derived components of the sleep period—REMS, N1, N2, N3, and WASO—expressed as proportions of the sleep period. Data expressed as a proportion or percent of a whole are called "compositional data" and require special statistical consideration [22, 34]. Because proportions are constrained to lie between 0 and 1, any distributional assumption of normality is violated. Moreover, because of the constraint that proportions must sum to one, any one variable is completely determined by the remaining variables (technically, the covariance matrix is singular). Such data are not suitable for standard factor analytic methods. A traditional solution is to exclude one variable, but this is problematic in that different relationships may appear between any two variables depending on which of the others is left out, and the solution will be susceptible to spurious correlations [35]. A technique for factor analyzing compositional data has been developed which produces interpretable factor loadings using all components of the composition [36]. The procedure works by applying logratio transformations to normalize the data and transform it to ordinary (Euclidean) coordinate space [22]. The transformed variables are then analyzed using standard factor analysis methods, with the resulting variables expressed as the log of the ratio of each original variable with the geometric mean of all variables (so-called CLR, or centered log ratio, coordinates [34]). The interpretation of each component variable, for example, REMS duration, is in terms of its relative contribution to the whole, rather than its absolute magnitude. The compositional factor analysis was conducted using the "pfa" function in the R package "robCompositions" [37, 38].

Statistical methods

Data were analyzed using linear regression modeling (Hypotheses 1, 2a–b, 3a–b, 4a–b, 5a–b) and linear mixed models for repeated measures (Hypotheses 2c, 4c, and 5c). See Table 2 for a description of the statistical model used to test each hypothesis. Linear mixed models included subjects as a random factor to accommodate repeated extinction trials within subjects. For characterizing extinction over time, several nonlinear functional forms were considered, including quadratic fits and piecewise linear fits, but a simple linear slope yielded the best fit in terms of standard AIC (Akaike Information Criterion) and BIC (Bayesian Information Criterion) information criteria. A random effect for time (block) was considered, but it correlated almost perfectly ($r = -.97$) with the random subject-specific intercepts, making it a redundant parameter. Therefore, time was modeled as a fixed effect. Models were fitted using the "mixed" command in Stata 16 [39]. No p-value adjustments were made for prespecified hypotheses.

Assumptions of linearity and normality of residuals were checked and found to be adequate. REMS duration was markedly skewed (skewness = 1.9), but models with REMS duration as the outcome nonetheless showed reasonably normal residuals due to the inclusion of control-nap REMS as a covariate. We conducted a sensitivity analysis using log-transformed REMS durations for adjusted and unadjusted models, and found no appreciable difference in results, and therefore present results for the untransformed data.

Exploratory analyses were conducted to determine whether any findings with REMS and sleep quality were moderated by sex and/or PTSD symptom severity (CAPS total score) by adding sex, CAPS total score, and all interactions to the linear regression and mixed model analyses described above. Exploratory correlational analyses were performed to explore bivariate relationships between standard PSG sleep and fear learning variables. p-values for exploratory correlations were Bonferroni corrected.

One participant from 47 initially enrolled had unusable data due to technical problems in both sleep and learning procedures and was excluded from the sample. All available data were used

Table 2. Statistical models for hypothesis testing and exploratory analyses

Hypothesis	Model	Outcome	Predictors
H1	LM	REMS duration in stress condition	Safety signal learning*, REMS duration in control condition
H2a	LM	Safety signal retention	REMS duration in stress condition*, REMS duration in control condition
H2b	LM	Differential conditioning retention	REMS duration in stress condition*, REMS duration in control condition
H2c	LMM	Conditioned response during extinction	REMS duration in stress condition, trial block, REMS duration × trial block interaction*, REMS duration in control condition
H3a	LM	Sleep quality during stress condition	Fear potentiation*, sleep quality during control condition
H3b	LM	Sleep quality during stress condition	Safety signal learning*, sleep quality during control condition
H4a	LM	Safety signal retention	Sleep quality during stress condition*, sleep quality during control condition
H4b	LM	Differential conditioning retention	Sleep quality during stress condition*, sleep quality during control condition
H4c	LMM	Conditioned response during extinction	Sleep quality during stress condition, trial block, sleep quality × trial block interaction*, sleep quality during control condition
H5a	LM	Fear potentiation	PTSD symptom level (CAPS scores)*
H5b	LM	Safety signal learning	PTSD Symptom Level (CAPS scores)*
H5c	LMM	Conditioned response during extinction	PTSD Symptom Level (CAPS scores)*

LM, ordinary least squares linear regression model; LMM, linear mixed effects regression model.
*Indicates key predictor variable for test of hypothesis.

in each analysis, with N ranging from 42 to 46 due to missing data described below.

Results

Table 3 depicts the demographic and clinical characteristics of the sample (N = 46). PTSD-positive subjects are those meeting current CAPS criteria for a PTSD diagnosis, while PTSD-negative subjects are those with no lifetime history of full-criterion PTSD after trauma exposure. PTSD in remission are subjects who previously met the criteria for PTSD but are currently in partial or full remission. CAPS scores reflect current CAPS scores. Mean CAPS score differed across PTSD groups ($F(2,43) = 38.04$, $p < .0001$), with PTSD group accounting for 64% of the variance in CAPS scores. Table 4 depicts the nap sleep characteristics of the entire sample (N = 46). One control-condition nap was excluded due to technical issues during data collection. All participants slept during the nap opportunity, with an average of 87 min (SD 30 m, range 6–118) including all naps. While sleep during the adaptation nap predictably differed from sleep in the stress and control nap conditions, there were no significant differences between nap sleep measures in the stress and control conditions. Of note, while the recording duration (lights out to termination of nap opportunity) ranged between 117 and 120 min in the control and stress conditions, a few subjects had shorter recording durations in the adaptation nap, as a result of subject-specific challenges with hook-ups that were addressed prior to subsequent visits. Thirty-five participants had REMS during the

Table 3. Demographic and clinical characteristics of the sample

	Combined sample (n = 46)	PTSD+ (n = 15)	PTSD– (n = 13)	PTSD remission (n = 18)
Age, mean (SD)	33.2 (7.0)	32.9 (5.4)	32.5 (7.3)	33.8 (8.1)
Biological sex (n, %)				
Female	22 (47.8)	10 (66.7)	7 (53.8)	5 (27.8)
Male	24 (52.2)	5 (33.3)	6 (46.2)	13 (72.2)
Education (n, %)				
High School	3 (6.5)	0 (0)	3 (23.1)	0 (0)
Some college	9 (19.6)	3 (20)	2 (15.4)	4 (22.2)
Bachelor's or associate degree	22 (47.8)	7 (46.7)	6 (46.2)	9 (50)
Post-graduate	12 (26.1)	5 (33.3)	2 (15.4)	5 (27.8)
Race (n, %)				
White	16 (34.8)	5 (33.3)	6 (46.2)	5 (27.8)
African-American	6 (13)	1 (6.7)	2 (15.4)	3 (16.7)
Other	24 (52.2)	9 (60)	5 (38.5)	10 (55.6)
Ethnicity (n, %)				
Hispanic or Latino	8 (17.4)	5 (33.3)	0 (0)	3 (16.7)
Not Hispanic or Latino	38 (82.6)	10 (66.7)	13 (100)	15 (83.3)
Marital status (n, %)				
Single	24 (52.2)	8 (53.3)	8 (61.5)	8 (44.4)
Married	10 (21.7)	3 (20)	2 (15.4)	5 (27.8)
Divorced	5 (10.9)	3 (20)	2 (15.4)	0 (0)
Separated	2 (4.3)	1 (6.7)	0 (0)	1 (5.6)
Other	5 (10.9)	0 (0)	1 (7.7)	4 (22.2)
CAPS score, mean (SD)	15 (10.4)	26.1 (7.5)	6.0 (5.1)	11.0 (6.3)

"PTSD+" subjects currently satisfy CAPS PTSD diagnosis; "PTSD–" subjects have no lifetime history of PTSD based on CAPS interview; "PTSD-in-remission" subjects previously, but not currently, met full-criteria for a CAPS PTSD diagnosis.

Table 4. Nap sleep characteristics of the sample

	Condition			Contrasts		
Sleep Variable	Adaptation nap mean (SD)	Control nap mean (SD)	Stress nap mean (SD)	Adaptation versus control (n = 45)	Adaptation versus stress (n = 46)	Control versus stress (n = 45)
Time in bed (TIB, min)	118.9 (3.5)	119.8 (0.7)	119.9 (0.3)	$t = 2.08$, $p = .040$	$t = 2.38$, $p = .020$	$t = 0.28$, $p = .777$
Total sleep time (TST, min)	78.6 (31.9)	94.4 (24.7)	88.9 (31.3)	$t = 3.53$, $p = .001$	$t = 2.26$, $p = .026$	$t = -1.29$, $p = .202$
Sleep latency (SL, min)	10.2 (12.8)	5.7 (4.7)	5.5 (6.5)	$t = -3.24$, $p = .002$	$t = -3.40$, $p = .001$	$t = -0.14$, $p = .886$
Wake after sleep onset (WASO, min)	16.0 (18.1)	12.4 (14.3)	16.3 (18.5)	$t = -1.04$, $p = .299$	$t = 0.09$, $p = .930$	$t = 1.13$, $p = .261$
Sleep maintenance (SM, %)	76 (15)	77 (13)	73 (21)	$t = 0.21$, $p = .832$	$t = -0.94$, $p = .351$	$t = -1.14$, $p = .255$
N1 duration (min)	13.2 (7.5)	11.0 (5.5)	10.8 (5.9)	$t = -2.46$, $p = .016$	$t = -2.65$, $p = .009$	$t = -0.17$, $p = .867$
N2 duration (min)	41.8 (22.6)	47.6 (16.1)	45.6 (22.1)	$t = 1.80$, $p = .075$	$t = 1.11$, $p = .271$	$t = -.070$, $p = .486$
N3 duration (min)	15.7 (15.4)	23.5 (14.9)	22.7 (18.3)	$t = 3.30$, $p = .001$	$t = 2.98$, $p = .004$	$t = -0.34$, $p = .734$
REMS duration (min)	7.9 (9.6)	12.3 (10.9)	9.9 (11.0)	$t = 2.48$, $p = .015$	$t = 1.11$, $p = .268$	$t = -1.38$, $p = .172$
REMS percent (REMS% = REMS min/TST ×100)	7.6 (9.2)	11.8 (10.3)	9.6 (10.2)	$t = 2.41$, $p = .018$	$t = 1.16$, $p = .248$	$t = -1.25$, $p = .214$

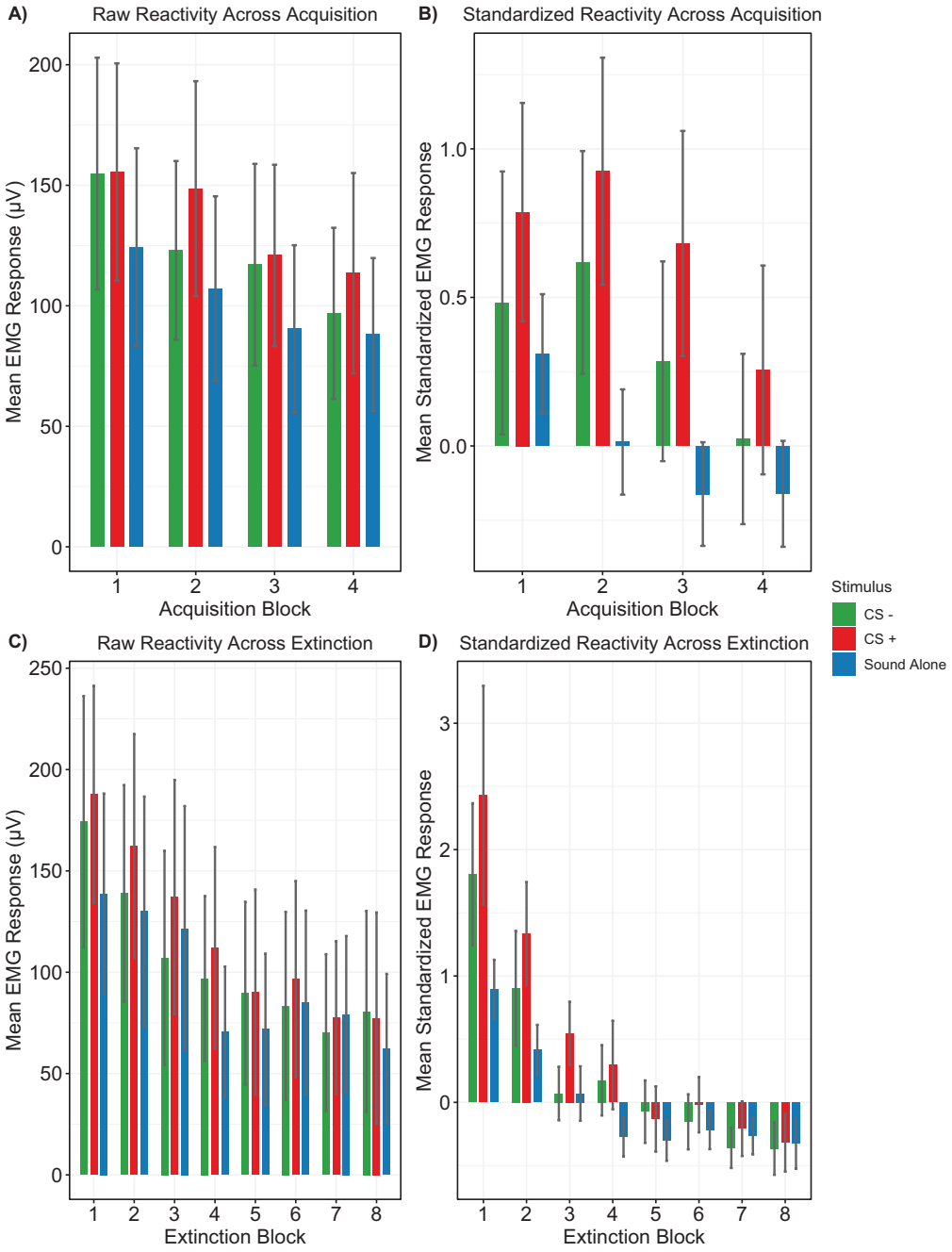


Figure 4. Fear-potentiated startle magnitude during acquisition and extinction. EMG startle magnitude during acquisition phase (A, B) and extinction (C, D). For acquisition, 44 subjects contributed data to the analyses. Acquisition and extinction EMG responses were standardized by subject-specific means and standard deviations of NA responses in acquisition and extinction sessions separately, and therefore are not directly comparable. For retention and extinction, 42 subjects contributed data to the analyses. Error bars are 95% confidence intervals.

control condition and 32 had REMS during the stress condition, with 26 having REMS during both the control and stress conditions. Six participants showed no REMS in either the control or stress condition nap.

Figure 4 depicts baseline-noise-corrected and standardized scores for startle EMG responses in both acquisition (Figure 4, A and B) and extinction (Figure 4, C and D) sessions. Two participants did not have usable acquisition data due to technical problems, and two additional subjects opted not to participate in the extinction session. In acquisition, a gradual decline in startle reactivity was observed for all stimulus types, an indicator of habituation over the course of the acquisition session. On average, extinction appears to occur before the end of the extinction session (see Figure 4, C and D). For this reason, we measured the slope of extinction over the first two blocks as well as over all eight blocks as planned.

Hypothesis 1: Does Safety Signal Learning Predict REMS Duration?

In support of Hypothesis 1, the linear regression model showed a positive effect of safety learning on REMS duration in the stress nap adjusted for REMS duration in the control nap (β = .43 [.14, .71], $t(40)$ = 2.99, p = .005; see Figure 5). The same

relationship was seen with unadjusted REMS duration (β = . 38 [.09, .67], $t(42) = 2.66$, $p = .011$).

Hypothesis 2a: Does REMS Duration Predict Retention of Safety Signal Learning?

There was no support for Hypothesis 2a; the linear regression model showed that control-nap adjusted-REMS duration was weakly associated with *higher* rather than lower response to the safety signal in block 1 of extinction, controlling for the safety signal in block 4 of acquisition (β = .25 [–.05, .54], $t(39) = 1.69$, $p = .099$).

Hypothesis 2b: Does REMS Duration Predict Retention of Differential Conditioning?

Contrary to Hypothesis 2b, the linear regression model showed no evidence of a REMS relationship with retention of differential conditioning (β = –.05 [–.38, .27], $t(39) = –0.033$, $p = .744$).

Hypothesis 2c: Does REMS Duration Predict More Rapid Extinction on Conditioned Responses?

Consistent with Hypothesis 2c, REMS duration during the stress condition, adjusting for control-nap REMS duration, was positively related to extinction rate over all eight blocks (greater REMS duration was associated with a steeper decline of conditioned responses). This is indicated by the statistically significant interaction between REMS and block in the linear mixed model (β = –.07 [–.14, –.01], $z = 2.10$ ($N = 42$), $p = .035$). The effect was similar for the model that did not adjust for control-nap REMS duration, β = –.07 [–.14, –.004], $z = 2.02$ ($N = 42$), $p = .037$. While the linear model provided a good statistical fit for the slope of extinction over time, we plotted the predicted scores separately for each block to examine changes in slope that may be obscured by the linear analysis (see Figure 6). For ease of visualization the continuous REMS duration is represented in the figure by discrete values of ± 1 SD from the mean, showing changes in response over blocks for subjects with relatively high and low REMS durations. A more rapid decline for high REMS subjects was apparent when considering just the change from block 1 to block 2 (REMS by block interaction effect β = –.28 [–.52, –.03], $z = 2.17$ ($N = 41$), $p = .030$). Overall, these findings indicate that the effect of REMS duration on extinction was concentrated to effects in early extinction from block 1 to block 2.

Integrated PSG sleep measure

To address Hypotheses 3a–b and 4a–c, compositional factor analysis was performed on data from the 45 participants with control nap sleep data. It indicated that 50% of the total variance was shared among the PSG components, and could be accounted for by two factors. We chose a one-factor solution over the two-factor solution because the factor accounted for 68% of shared variance, it involved appreciable loadings from all five sleep-period components, and showed a reasonably interpretable factor loading pattern (see Table 5). WASO and N1 showed strong negative loadings of –.78 and –.63, respectively, while REM and N3 had positive loadings of .46 and .59,

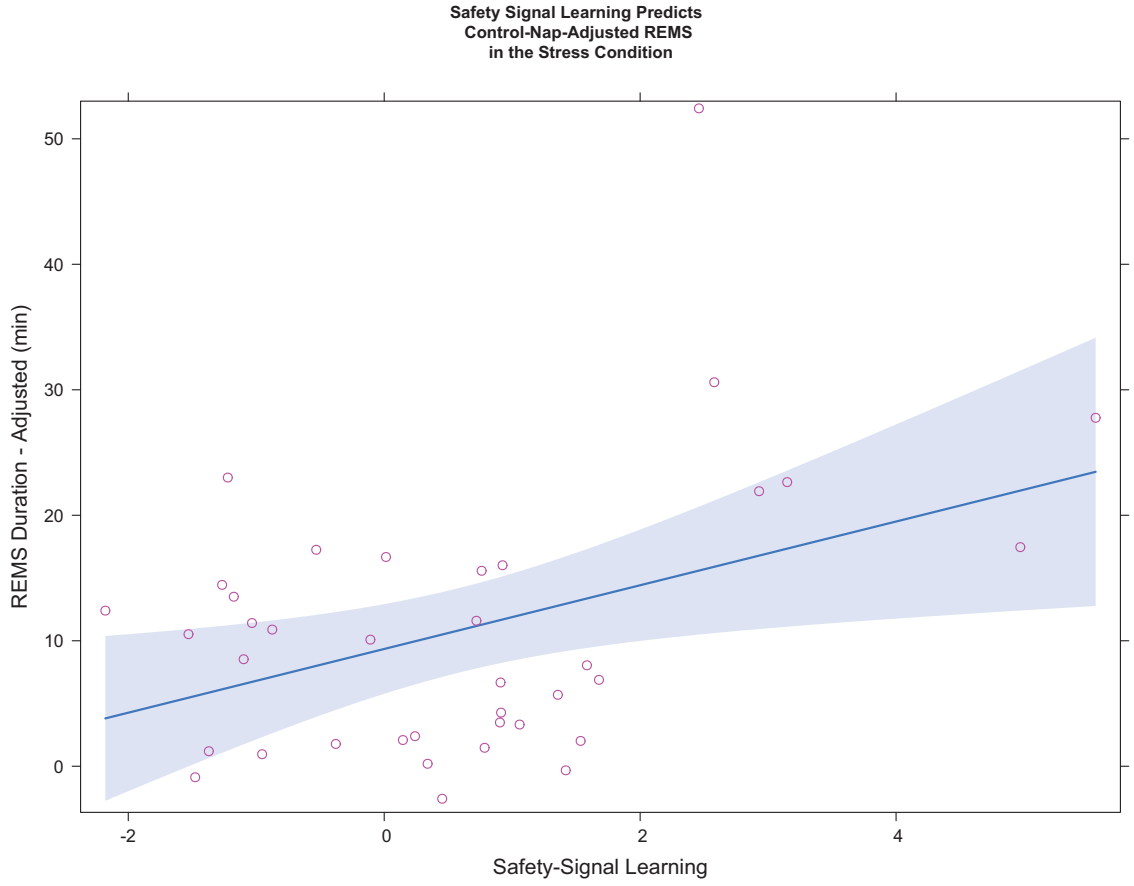


Figure 5. Safety signal learning predicts REMS duration in the stress condition, adjusted for control-condition REMS duration ($n = 44$). Error band represents 95% confidence interval.

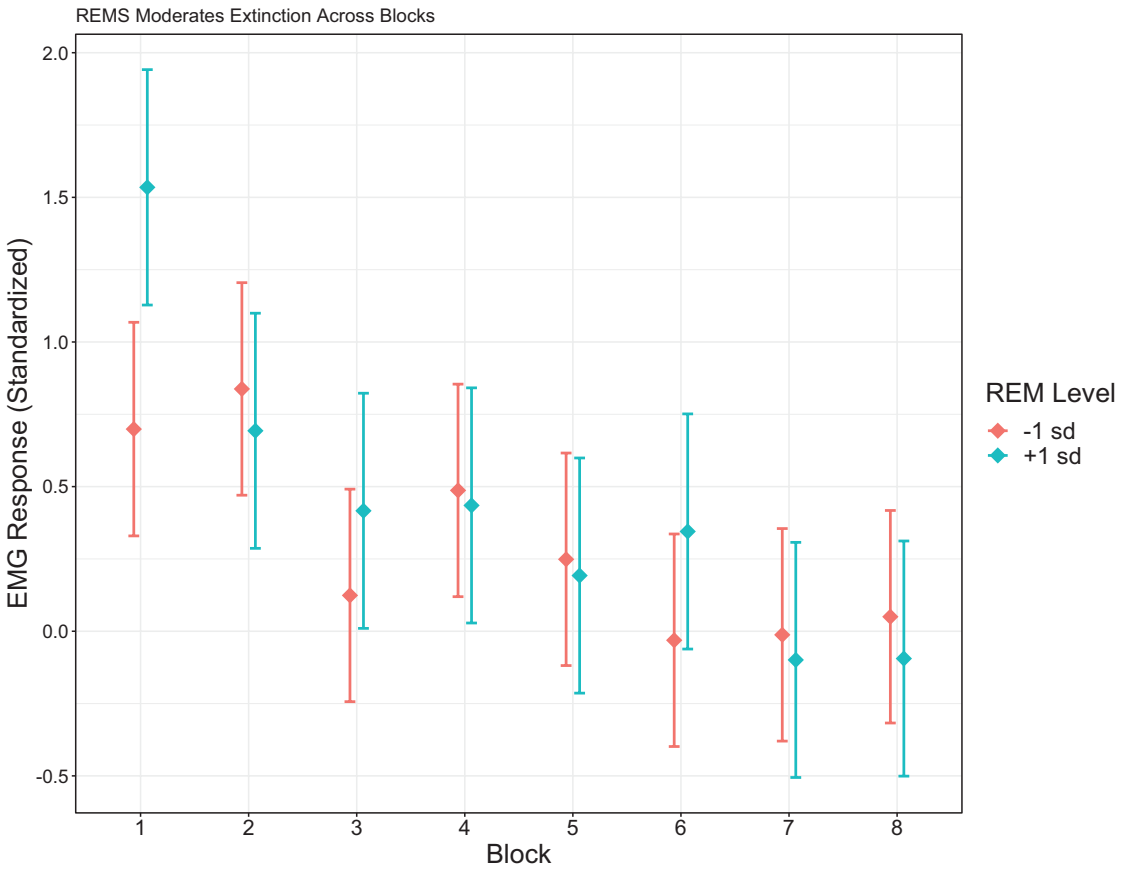


Figure 6. Responses over blocks of extinction for subjects with high and low REMS durations ($n = 42$). Here, REMS duration in the stress condition is adjusted for control-nap REMS duration. Higher adjusted REMS duration is associated with higher intercept and steeper decline over blocks. The statistical model fitted a linear slope over blocks (see main text). Shown here are responses predicted for each block separately and for values of REMS representing 1 s.d. above and below the mean for better visualization of data. Error bars are 95% confidence intervals.

Table 5. Factor loadings of PSG sleep period components on integrative sleep latent factor and proportions of unique variance

Component	Loading	Uniqueness
WASO	−0.781	0.366
N1	−0.633	0.492
N2	0.375	0.647
N3	0.587	0.525
REM	0.458	0.606

Uniqueness values are the proportion of variance of each component that is not shared with other components, and therefore not represented in the latent factor.

respectively. N2 loaded more weakly, but positively (.37), on this first factor. The factor seems to reflect the contrast between interrupted and light sleep on the one hand compared to deeper and REMS on the other, and we therefore provisionally interpret this integrative PSG measure as an overall PSG sleep quality score. We derived factor scores from the control-condition nap and applied the same scoring weights to the stress nap to provide a consistently scored sleep measure for both conditions. To lend validity to the measure as a possible reflection of sleep quality, we examined the relationship between the factor score and a three-level (poor, fair, good) self-report rating of sleep quality, obtained from all subjects at the end of each nap opportunity. The factor score was correlated with sleep rating ($r = .22$, $p = .042$), due primarily to its association with poor versus fair/good sleep ratings. In a logistic regression, factor scores predicted poor subjective sleep quality (standardized OR = .44, $z = -2.57$, $p = .010$).

Hypothesis 3a: Does Higher Fear Potentiation Predict a Reduced Balance of REM and N3 Relative to N1 and WASO?

We found no evidence for a relationship between fear potentiation and subsequent control-nap-adjusted stress-condition PSG sleep quality, as measured by the integrative latent factor score reflecting this REM/N3 versus N1/WASO balance (Hypothesis 3a; $\beta = -.08$ [−.36, .21], $t(40) = -0.53$, $p = .596$). The finding was similar for the model predicting unadjusted stress-condition PSG sleep quality ($\beta = -.03$ [−.34, .28], $t(41) = -0.22$, $p = .829$).

Hypothesis 3b: Does Lower Safety Signal Learning Predict a Reduced Balance of REM and N3 Relative to N1 and WASO?

Consistent with Hypothesis 3b, lower safety signal learning did predict poorer control-nap-adjusted stress-condition PSG sleep quality, as measured by the integrative latent factor reflecting REM/N3 versus N1/WASO balance (Hypothesis 3b, $\beta = .28$ [.005, .56], $t(40) = 2.05$, $p = .046$; Figure 7A). The same relationship was seen with unadjusted stress-condition PSG sleep quality ($\beta = .34$ [.05, .63], $t(41) = 2.34$, $p = .024$).

Hypothesis 4a: Does A Higher Balance of REM and N3 Relative to N1 and WASO Predict Greater Retention of Safety Learning?

Contrary to Hypothesis 4a, greater stress-condition PSG sleep quality predicted *higher* safety signal response at block 1 of extinction, adjusting for block 4 of acquisition ($\beta = .34$ [.04, .64], $t(39) = 2.26$, $p = .030$).

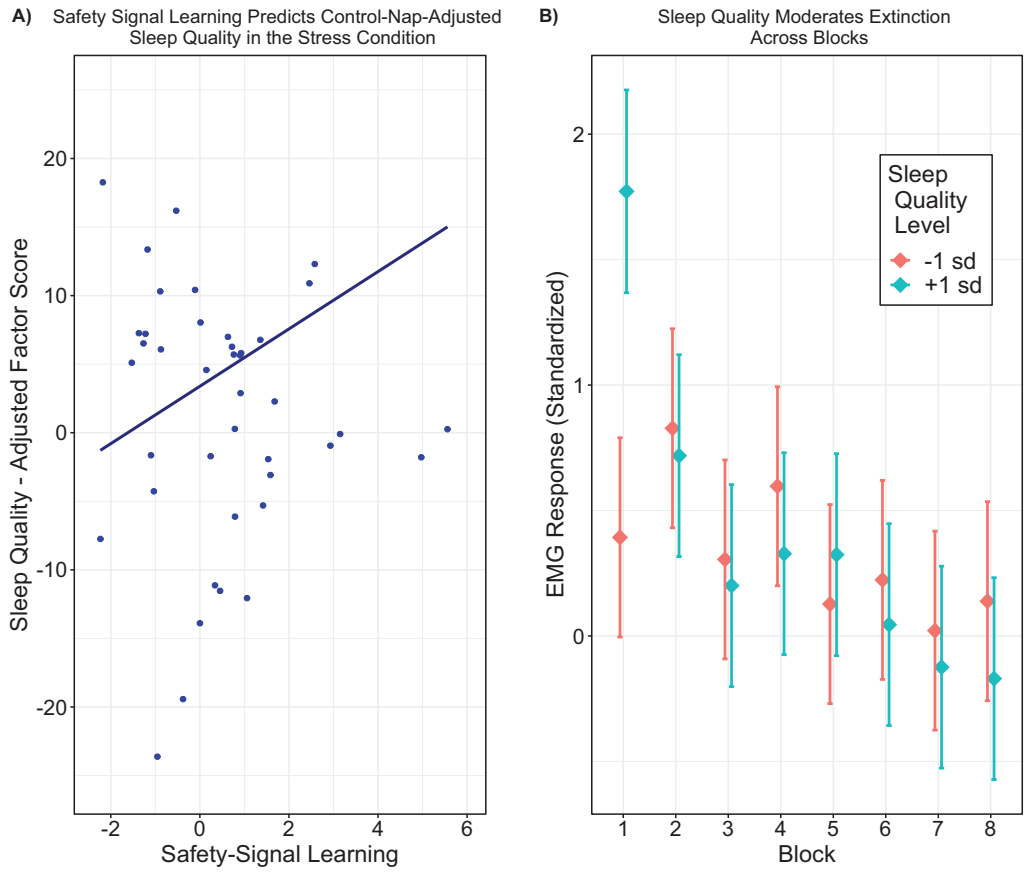


Figure 7. Relationship of integrative PSG sleep variable ("PSG sleep quality") and emotional learning. (A) Safety learning predicts PSG sleep quality, adjusted for control-condition PSG sleep quality ($n = 44$). (B) Predicted responses over blocks of extinction for subjects with high and low PSG sleep quality ($n = 42$). Here, PSG sleep quality in the stress condition is adjusted for control-nap PSG sleep quality. Higher adjusted PSG sleep quality is associated with higher intercept and steeper decline over blocks. For (B), the statistical model fitted a linear slope over blocks (see main text). Shown here for (B) are responses predicted for each block separately for better visualization of data. Error bars are 95% confidence intervals.

Hypothesis 4b: Does A Higher Balance of REM and N3 relative to N1 and WASO Predict Greater Retention of Differential Conditioning?

Contrary to Hypothesis 4b, the linear regression analysis did not demonstrate an effect of control-nap-adjusted PSG sleep quality on retention of differential conditioning (β = .14 [–.18, .47], $t(39) = 0.91$, $p = .368$).

Hypothesis 4c: Does A Higher Balance of REM and N3 Relative to N1 and WASO Predict More Rapid Extinction of the Conditioned Responses?

Consistent with Hypothesis 4c, mixed model analysis showed that PSG sleep quality was associated with more rapid extinction of conditioned responses. This is indicated by the statistically significant interaction of PSG sleep quality and block in the linear mixed model (β = –.05 [–.08, –.02], $z = -3.33$, $p = .001$), suggesting that extinction is steeper (more rapid) for those with higher sleep quality. The decline was also steeper over the first two blocks (linear mixed model block by PSG sleep quality interaction β = –.40 [–.65, –.16], $z = -3.24$, $p = .001$). Figure 7B shows extinction over blocks for subjects with high and low PSG sleep quality. As in Figure 6, scores are predicted separately for each block to show changes in slope that may be obscured by the linear analysis, and, for ease of visualization, the continuous PSG sleep quality score is represented by discrete values of ± 1 standard deviation from the mean, representing high and low values of sleep quality.

Hypothesis 5a: Does higher CAPS-measured PTSD severity predict greater fear potentiation? Contrary to expectations, PTSD symptom severity was not significantly associated with fear potentiation in the linear regression model (β = –.22 [–.52, .08], $t(42) = -1.46$, $p = .114$).

Hypothesis 5b: Does higher CAPS-measured PTSD severity predict reduced safety signal learning?

Additionally, CAPS-measured PTSD severity did not show a relationship with safety signal learning in the linear regression model (β = .20 [–.17, .45], $t(42) = 0.91$, $p = .370$).

Hypothesis 5c: Does higher CAPS-measured PTSD severity predict poorer extinction of conditioned responses?

Contrary to our hypothesis, CAPS scores did predict extinction rate over 8 blocks, but the relationship was opposite to the hypothesized effect: Higher CAPS scores were associated with steeper extinction (β = –.03 [–.06, –.002], $z = -2.12$, $p = .034$). However, this effect was not statistically significant over the first two blocks (β = –.04 [–.30, .22], $z = -0.31$, $p = .757$). This is depicted in Figure 8.

Exploratory Analyses: Does biological sex or PTSD severity moderate relationships between fear learning and sleep?

We conducted exploratory analyses to examine whether the findings with REMS and sleep quality were moderated by sex and/or PTSD symptom severity (CAPS total score) by adding sex, CAPS total score, and all interactions to the linear regression and mixed model analyses described above. There were no

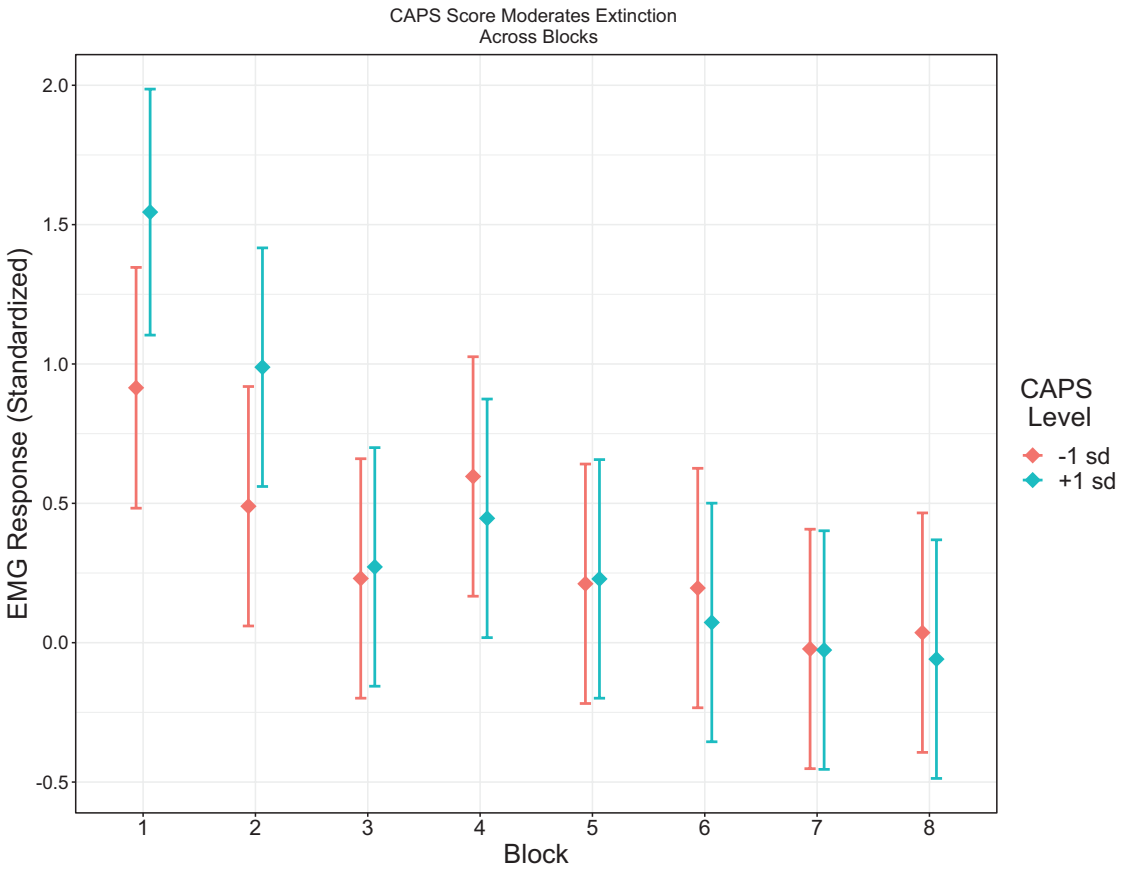


Figure 8. Predicted responses over blocks of extinction for subjects with high and low CAPS total score ($n = 42$). The statistical model fitted a linear slope over blocks (see main text). Shown here are responses predicted for each block separately for better visualization of data. Error bars are 95% confidence intervals.

significant main effects of sex in the regression models, but one significant interaction between sex and safety signal learning in predicting REMS duration in the stress nap (sex by safety signal learning interaction: β = −.86 [−1.53, −.18], $t(34) = -2.58$, $p = .014$). Testing the safety signal learning effect within each sex shows that the relationship of safety learning with control-nap-adjusted REMS duration reported for Hypothesis 1 is due almost exclusively to the effect in males (see Figure 9): For men, β = .55 [.22, .88], $t(34) = -3.36$, $p = .002$; for women, β = −.04 [−.60, .51], $t(34) = -0.16$, $p = .874$. A sex by safety learning interaction of approximately the same magnitude was observed in predicting unadjusted stress-condition REMS duration (interaction β = −.82 [−1.50, −.18], $t(36) = -2.43$, $p = .020$). On the other hand, there were no moderating effects of CAPS scores on the REMS and PSG sleep quality findings.

In the absence of any effects of CAPS scores on safety signal learning and fear potentiation variables in acquisition, we examined the relationship between learning variables and CAPS hyperarousal and intrusion subscales in post-hoc analyses. Phenomena including hyperactive startle and physiological reactivity to stressful stimuli fall into these categories such that examining these subscales separately might be more revealing. There were no significant effects on acquisition learning variables, but there was a relationship between higher hyperarousal score and poorer safety signal retention, (β = .33 [.02, .64], $z = 2.14$ ($N = 42$), $p = .039$). There was also a significant effect of hyperarousal on the slope of extinction, analogous to that seem with the total CAPS score (interaction β = −.03 [−.06, −.004], $z = -2.21$ ($N = 42$), $p = .027$). This suggests that the total CAPS effect may be explained by the hyperarousal symptoms.

Additionally, we present a correlation analysis to explore relationships between standard PSG sleep measures and learning measures in the stress condition (Supplementary Table S1). There was a significant Spearman correlation between TST and extinction slope (rho = −.53, Bonferroni corrected $p = .011$).

Discussion

The above-described findings provide new evidence that associative fear learning impacts subsequent sleep, and that sleep impacts subsequent fear information processing. First, this study strengthens the evidence that greater safety signal learning enhances REMS, and that increased REMS enhances the rate of extinction of conditioned responses. There is now compelling evidence that REMS is important for processing emotionally salient information [9, 40] and some evidence that disrupted and/or reduced REMS contributes to adverse mental health outcomes after stress [12, 41]. These findings provide additional evidence that (laboratory) stressors impact REMS quantities and that these quantities in turn affect emotional information processing. A relationship between greater safety signal learning and longer REMS was observed with and without controlling for control-nap REMS, but the observation of this relationship in the controlled analysis lends greater strength to the argument that there is a causal relationship between the magnitude of

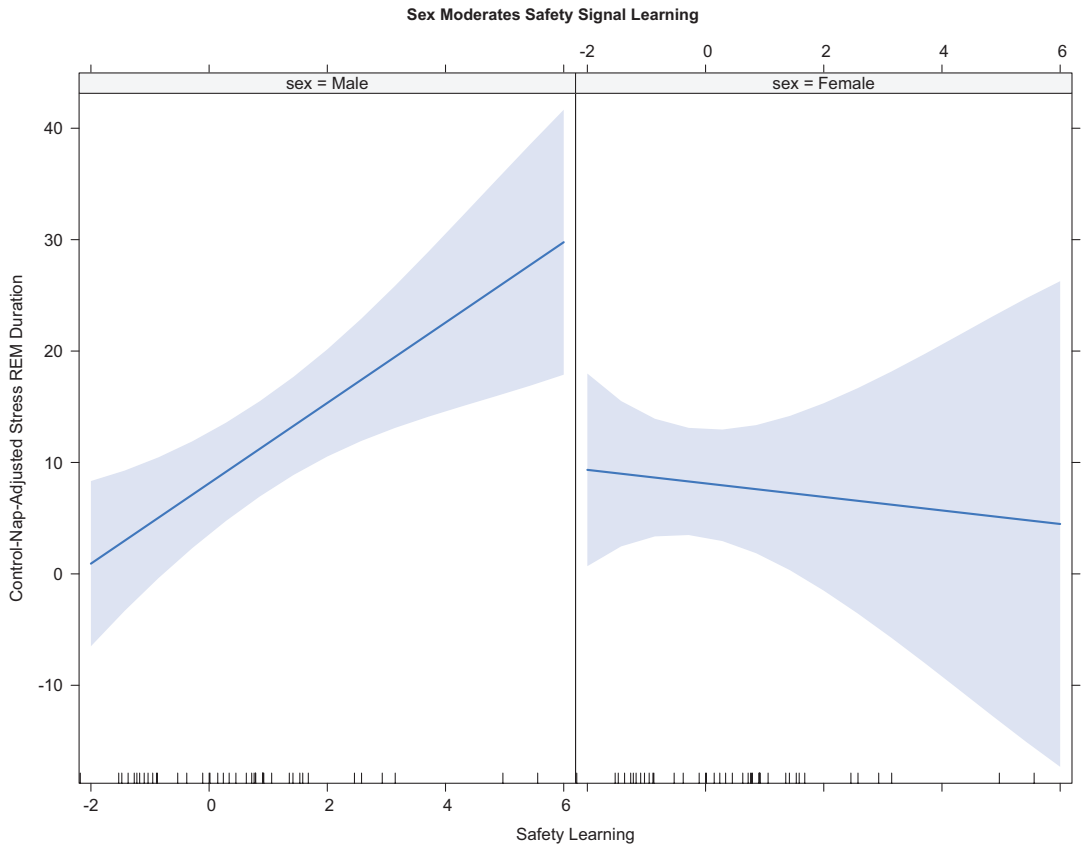


Figure 9. Safety signal learning effect on subsequent REMS is accounted for by effect in males only (n = 44). Error band represents 95% confidence interval.

the safety signal learning response and a change in subsequent REMS, and between the quantity of REMS change and subsequent processing. Nonetheless, this study cannot rule out that an unknown underlying factor contributes to both increased safety learning and increased REMS in the context of a stressor. Creative approaches for manipulating safety learning prior to sleep measurement would be needed to more directly demonstrate a causal relationship. In the absence of effective manipulations of safety signal learning both in the laboratory and in clinical practice, further research into the biological underpinnings of safety signal learning is needed to determine whether those biological substrates can be directly targeted.

Despite evidence of a sleep pathway linking safety learning and post-sleep information processing, support for our expectations with respect to retention and extinction of conditioned responses was mixed. *Contrary* to expectations, we observed that greater post-conditioning REMS was associated with a *higher* safety signal response (trend effect) at the beginning of extinction. A decrease in this response, controlling for presleep response, would be more consistent with retention of safety learning. Our extinction analyses also showed that greater post-conditioning REMS was associated with higher combined conditioned response (CS+ and CS–) in block 1 of extinction. Nonetheless, we *did* observe that a greater post-conditioning REMS duration resulted in a steeper slope of extinction of conditioned responses. This is consistent with some reports indicating REMS benefits fear extinction processes, even though most reports have observed effects at extinction recall (for which we do not have data) rather than at initial extinction learning [12, 42]. Additionally, we observed a relatively *precipitous* decline between block 1 and block 2 of extinction in association with higher REMS duration, with and without the control-nap REMS adjustment. Because most subjects extinguished by block 8, slope differences from block 1 to block 8 may in fact reflect the higher conditioned response in early extinction. The REMS by block interaction observed over the first two blocks, however, provides more support for the idea that REMS is associated with a more efficient regulation of emotional responses after sleep. Altogether, these findings are suggestive that REMS enhances post-sleep reactivity to previously conditioned stimuli but also allows for rapid regulation of responses in the context of safety. Parallel effects were observed using our integrative PSG sleep measure.

Findings with respect to PTSD symptom severity, as measured by CAPS total score, were generally not consistent with expectations. We did not observe a relationship between PTSD symptoms and fear potentiation or safety signal learning. While the literature reports mixed results with respect to differences in initial fear potentiation, or "fear load," in PTSD versus controls, the expectation is nonetheless logical given that PTSD is characterized by hyperarousal, including an exaggerated startle response and strong physiological responses to stressful triggers. Safety signal learning is considered to be an adaptive learning process, and impaired safety signal learning has been observed in PTSD, and has been proposed to be a biomarker for the disorder [10, 28, 43]. Relationships were also not observed when the hyperarousal and intrusion clusters were examined separately, although exploratory analyses did reveal that greater

hyperarousal symptoms predicted poorer retention of safety signal learning. The paucity of PTSD effects is hard to explain, although it has been proposed that different maladaptive fear learning pathways may lead to the same outcome of PTSD [8, 44], reducing our ability to observe effects and explaining inconsistencies across studies. Interestingly, we did observe that higher CAPS score was associated with higher response to conditioned stimuli (both CS+ and CS– combined) at the beginning of extinction in conjunction with a steeper slope of extinction as CAPS score increased. Hyperarousal symptoms alone showed a similar relationship. The overall CAPS finding parallels the REMS and PSG sleep quality relationships with conditioned responses (CS+ and CS– combined) in block 1 of extinction and with the slope of extinction from block 1 to block 8. While this commonality seems counterintuitive, it is likely that this is explained by the fact that most participants extinguished conditioned responses by block 8, such that a steeper linear slope of extinction essentially reflects the higher starting point. On the other hand, and in contrast to the observation that more REMS and higher magnitude of the integrative PSG sleep measure were linked to a more precipitous drop in conditioned responses from block 1 to block 2 of extinction, higher CAPS score did *not* show this effect between blocks 1 and 2 of extinction. This is suggestive that *both* higher PTSD symptoms and higher REMS and PSG sleep quality lead to higher (recall of) conditioned responses in early extinction but *only* REMS and higher PSG sleep quality lead to a robust and rapid reduction in the conditioned response in the safe (aversive-stimulus-free) context of the extinction session. In the overall model incorporating CAPS score and biological sex, we did not observe a statistically significant interaction between PTSD symptom severity and REMS on extinction from block 1 to block 2, although the effect was in the direction indicating that CAPS score could *reduce* the impact of prior REMS on early extinction. Larger sample sizes would be necessary to examine this interesting question further.

Our analysis derived an integrative PSG sleep variable based on factor analysis of the main sleep-stage and wake components of the entire sleep period spanning from sleep onset to final awakening, which explained 68% of the shared variance of these variables. Interestingly, this variable demonstrated a similar relationship with fear learning processes as did REMS, even though durations of sleep stages N1 and N3, and WASO, loaded more highly onto this variable than did REMS. Furthermore, factor analysis demonstrated that REMS had a large amount of unique variance (i.e. variance not explained by this variable; see Table 5). The effects of this variable, therefore, are unlikely to be due entirely to the effects of REMS. Growing research indicates that both REMS and NREMS stages may be important for adaptive emotional information processing, however, no studies of which we are aware describe and use in their analysis a PSG sleep variable that integrates contributions of all the sleep-stage and intrusive wake components of the sleep period. A novel approach involving compositional data analysis, already implemented in research on sleep vs. wake activity over the 24-hour day [35], fulfills assumptions of normality and orthogonality of dimensions required to perform a factor analysis using a traditional approach. In the current study, the relative loadings of WASO and N1 versus N3 and REMS on this variable and its predictive value with respect to poor versus fair/good subjective nap sleep quality are indicators that this variable taps into the concept of sleep quality and *also* predicts neurobiological measures of emotional learning. The approach introduced here may therefore have value in linking psychological, behavioral, and neurobiological outcomes with an integrative PSG measure of sleep quality.

Last but not least, our exploration of biological sex effects yielded compelling results with respect to sexual dimorphism in the sleep and emotion processing relationship. Similar to findings from a recent meta-analysis, we discovered that effects with respect to REMS and safety signal learning were explained by a robust effect in males and absence of (or possibly an opposite direction) effect in females. The results are suggestive that males mount a REMS response with increased safety signal learning, which may be associated with benefits for post-sleep emotional information processing, but females do not. The interaction effect for sex was not observed in analyses involving the integrative PSG sleep quality variable, although results were in the same direction. This adds to the evidence indicating that examination of biological sex effects is critical for understanding fear learning and sleep, and raises the possibility that therapeutics targeting sleep in males may be different from those indicated in females. While some studies carefully control for menstrual cycle phase in measurement of sleep and/or fear learning [17], this was beyond the scope of the current study, in which participants were studied over several weeks and at different phases of the menstrual cycle. Comparisons between male subjects and females at different phases of the cycle is critical to determine whether biological sex and/or hormonal milieu impact sleep and emotional-learning relationships.

Limitations

This study is the largest study of sleep and fear learning in trauma subjects of which we are aware, but several factors limit conclusions that can be drawn. While this study's sample size is impressive relative to existing studies, it is still suboptimally powered when considering the relationships and interaction effects of interest here. The possibilities of Type I and Type II errors need to be considered as alternative explanations for our findings, and this underscores the importance of well-powered studies to advance science in this area [45–47]. Additionally, the current experiment was conducted in the context of a larger experiment on emotional memory and sleep. Participants experienced another potentially stressful protocol after the fear acquisition protocol and prior to nap sleep, and another potentially stressful protocol after nap sleep but prior to startle retention and extinction procedures. Although we propose that the additional morning protocol may have served to amplify pre-nap stress effects on sleep, the post-nap protocol may have reduced observable impacts of nap sleep on retention and extinction. On the other hand, all participants were exposed to exactly the same procedures, therefore, if any noise was introduced into the protocol, it was standardized across participants. Additionally, while a handful of studies now provide evidence that nap sleep studies can contribute insights into sleep-dependent emotional information processing [42, 48–50], nap sleep effects may differ from overnight sleep effects in ways that cannot be studied here. Nonetheless, our observation of significant effects with REMS and with a novel integrative measure of PSG sleep, seen even when controlling for sleep in a control-condition nap, enhances our confidence in the effects of even small quantities of sleep in the relationships of interest.

In summary, the above findings enhance our confidence in a relationship between REMS and emotional learning and suggest a novel, though still exploratory, approach for examining an integrative PSG sleep quality relationship with emotional learning. Furthermore, they highlight the critical importance of examining biological sex effects in these relationships. More research is needed to better understand the complex relationship between PTSD and these fear learning processes, and the role of sleep in these relationships.

Supplementary Material

Supplementary material is available at *SLEEP* online.

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Disclosure Statement

None declared.

Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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