Beta spectral power during sleep is associated with impaired recall of extinguished fear

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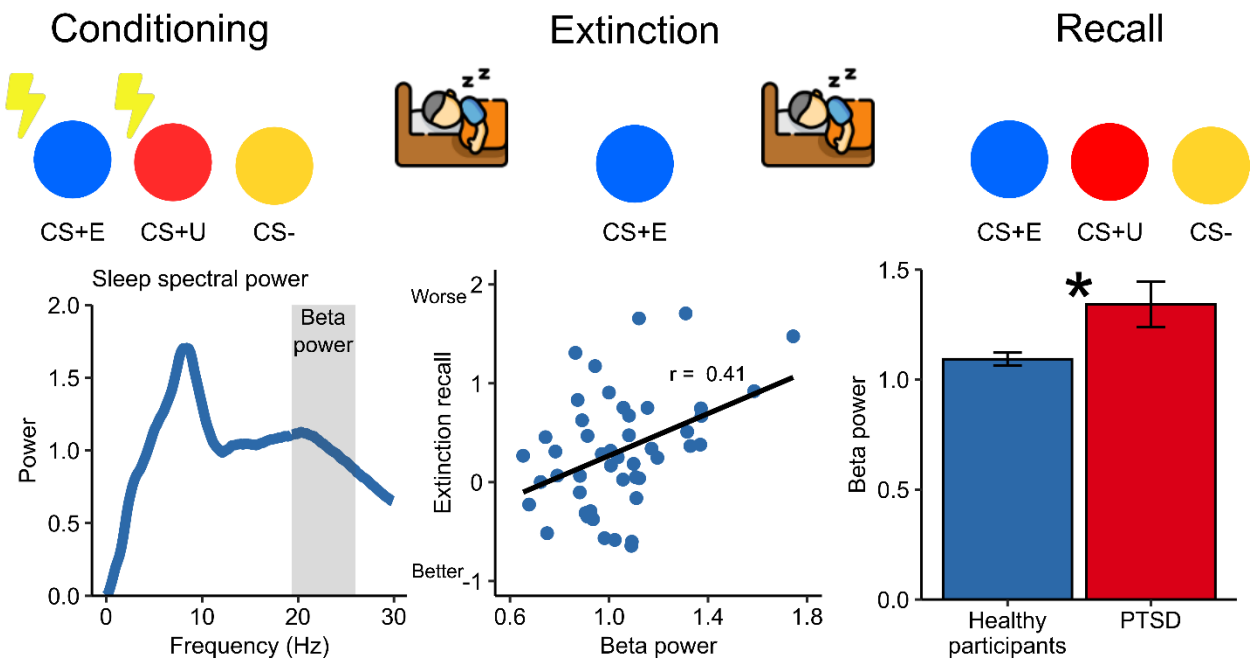
Abstract

The failure to retain memory for extinguished fear plays a major role in the maintenance of post-traumatic stress disorder (PTSD), with successful extinction recall necessary for symptom reduction. Disturbed sleep, a hallmark symptom of PTSD, impairs fear extinction recall. However, our understanding of the electrophysiological mechanisms underpinning sleep's role in extinction retention remain underdetermined. We examined the relationship between the microarchitecture of sleep and extinction recall in healthy humans (n=71, both male and females included) and a pilot study in individuals with PTSD (n=12). Participants underwent a fear conditioning and extinction protocol over two days, with sleep recording occurring between conditioning and extinction. Twenty-four hours after extinction learning, participants underwent extinction recall. Power spectral density (PSD) was computed for pre- and post-extinction learning sleep. Increased beta band PSD (~17-26Hz) during pre-extinction learning sleep was associated with worse extinction recall in healthy participants (r=.41, p=.004). Beta PSD was highly stable across three nights of sleep (intraclass correlations (ICC)>0.92). Results suggest beta band PSD is elevated in PTSD, and is specifically implicated in difficulties recalling extinguished fear.

Keywords: fear conditioning, extinction, memory, PTSD, beta power

Graphical abstract



Significance statement

Disturbed sleep is a hallmark feature of posttraumatic stress disorder (PTSD). Certain neural oscillations that occur during sleep have been shown to be altered in PTSD. These include increased 15-30Hz beta oscillations, which are believed to index cortical hyperarousal. Alongside sleep disturbances, patients also show difficulty in recalling extinguished fear. Although prior studies have linked sleep with extinction retention, no studies have investigated the role that neural oscillations during sleep play in this process. Here, we show in healthy participants that increased beta oscillatory power during sleep is associated with impaired extinction retention. Therefore, this study provides new evidence that electrophysiological changes in the sleep EEG of PTSD patients is also implicated in extinction recall processes.

Introduction

Post-traumatic stress disorder (PTSD) is an emotional disorder characterized by a hyperarousal syndrome that impacts both daytime functioning and sleep [1]. PTSD has been described as a disorder of emotional learning and memory that can be modeled experimentally using Pavlovian aversive conditioning paradigms [2–4]. Such paradigms pair aversive stimuli with neutral stimuli, generating a measurable conditioned fear response. This learning process parallels the maladaptive fear learning in PTSD, whereby neutral situations, locations or objects become associated with an aversive outcome (i.e., the index trauma). Fearful responses to non-threatening stimuli can be overcome by a process of extinction learning, whereby individuals are repeatedly presented with conditioned stimuli in the absence of the aversive outcome. Memory for learned extinction attenuates fear responses [4] and is necessary for PTSD symptom reduction [5].

Sleep disturbances are considered a hallmark figure of PTSD [6,7], and a growing body of research has linked sleep to extinction memory processes. Both rapid eye movement (REM) and non-REM (NREM) sleep following extinction learning has been linked to the consolidation and retention of extinction memory [8–14]. Additionally, total sleep deprivation *prior* to extinction learning can disrupt later recall of extinguished fear, suggesting that sleep prior to extinction learning may be important in preparing the brain for the consolidation of new learning [15].

Theta (~4-8Hz) spectral power during REM sleep has been linked to emotional memory processing [16], and has been shown to be elevated in trauma-exposed individuals who were resilient to, compared to those diagnosed with, PTSD [17]. Beta spectral power (~16-30Hz) has also been shown to be elevated in PTSD compared to those without PTSD [18–21], and has been theorized as a marker of disturbed sleep and hyperarousal [22–25]. Alongside these increases in high-frequency power, studies have also documented a reduction in low frequency power (<4 Hz) that is consistent with reduced slow wave sleep seen in patients [20,21,26]. These findings paint a picture of PTSD sleep being characterised by both a reduction in REM theta oscillations, potentially reducing emotional processing capabilities, as well as a reduction in restoration promoting low frequency oscillations and an increase in higher frequency signals, consistent with the idea of cortical hyperarousal during sleep. Although these signatures are potentially important biomarkers of PTSD, results are not always consistent across studies [27–29].

These literatures provide converging evidence for a critical role of sleep in fear extinction retention. In PTSD, sleep disturbances may play a key role in the difficulty for these individuals to retain memories of extinguished fear and thus maintain disordered symptoms. Here, we examined whether sleep oscillatory activity that is altered in PTSD, such as theta and beta spectral power, impact the basic fear memory processes that are implicated in PTSD development, such as the retention of extinction memories.

Utilizing an existing dataset, we first examined associations between pre-extinction learning sleep spectral power and extinction recall in healthy controls [15]. Given that total sleep deprivation prior to extinction learning impairs later recall, we reasoned that differences in sleep microarchitecture *during* sleep prior to extinction may also play a role in later retention. As a secondary analysis, we also examined associations with *post*-extinction learning sleep spectral power, on the basis of other work showing sleep macroarchitecture correlations [8,11–14]. Utilizing a second pilot dataset [30] we tested whether similar associations were present in a sample of individuals with PTSD. Finally, if these measures of sleep oscillatory activity reflect diagnostic biomarkers of PTSD, it is critical that they are consistently represented in the sleep record. As such, we conducted exploratory analyses examining the night-to-night consistency in potentially problematic spectral power identified through the primary analyses.

We predicted that in healthy controls, greater theta-band spectral activity during REM sleep would be associated with better extinction recall, given theta's putative role in emotional memory processing. Because sleep disruption via deprivation impairs extinction recall [15], and greater beta-band spectral power during sleep is a proxy of disturbed sleep [31], we expected greater beta-band spectral activity during sleep (both REM and NREM) to be associated with poorer subsequent recall of extinguished fear. In the pilot group of individuals with PTSD, we expected decreased REM theta and increased REM/NREM beta activity compared to the healthy controls. We also predicted that associations between spectral power and extinction recall would be of the same magnitude and direction as observed in the healthy controls.

Study 1

Methods

This study was a reanalysis of a previously published dataset [15,32]. Please see [15] for the full protocol details. The investigation was carried out in accordance with the Declaration of Helsinki. The University of California, San Diego's Human Research Protections Program approved the study. Informed consent was obtained from all participants.

Participants

Seventy-three healthy adults were enrolled in the study, and 71 participants with full datasets were included in the final analysis. Participants were healthy adults aged 18-39 years old (M = 24 years, SD = 5 years; 41% female) with no current medical or psychiatric diagnosis, and exhibited a consistent blink-startle response at screening (over 75% discernable responses to twelve 105dB 40ms startle pulses).

Procedure

Participants spent 4 consecutive days and nights in the sleep laboratory (**Figure 1A**). Following an adaptation night of sleep, participants underwent a fear potentiated startle procedure (see below) consisting of three sessions: fear acquisition (day 1); fear extinction learning (day 2); and fear extinction recall (day 3, the focus of the present analysis). All testing took place in the evening. Participants were randomized to either: 1) a Normal sleep condition consisting of a full night of sleep on all experimental nights (*n*=21); 2) 36 hours of total sleep deprivation after extinction learning ("Post-extinction deprivation", *n* = 25) or 3) 36 hours of total sleep deprivation before extinction learning ("Pre-extinction deprivation"; *n* = 25). Sleep was monitored using polysomnography (see below).

Fear potentiated startle

Each session began with six startle pulses (108db, 30ms acoustic startle probes) presented in the absence of any other stimuli in order for participants to acclimate startle responses. The fear acquisition session on day 1 consisted of 1) eight 6-second long presentations of a blue circle (CS+), following by a 500 ms electric shock (US) in 75% of blue circle trials; 2) eight 6-second presentations of a red circle serving as a second CS+, also followed by a 500 ms electric shock US in 75% of trials; 3) sixteen 6-second presentations of a yellow circle serving as a non-reinforced conditioned stimulus (CS-), never followed by a shock; and 4) sixteen presentations of the startle pulse in the absence of any stimuli (noise only; NA). The first half of the acquisition session consisted of only blue CS+ trials, and the second half consisted of only red CS+ trials. Startle pulses were presented 4 seconds following CS+ or CS- onset. Stimulus presentation was randomized within each CS+ acquisition block (blue vs red) and with the constraint of the two trials of each type (CS+, CS-, and NA) per block. On day 2, participants underwent extinction learning, consisting of 16 presentations of each stimulus type (blue CS+, yellow CS-, NA) in a block randomized order as in the acquisition session. No shocks were presented during this session. On day 3, participants completed extinction recall. This session was identical to the fear acquisition session except no shocks were delivered.

Fear extinction recall analysis

Eyeblink electromyogram (EMG) responses were recorded and used to index startle responses. Initial data reduction involved averaging responses to CS+ and CS- trials within each session into blocks of two trials each. NA trials within a session were averaged to acquire a mean startle response in the absence of any stimuli. This average was then subtracted from the respective CS+ and CS- block within each session, creating scores representing potentiated startle above the noise-alone condition for each CS type within each block [15,32–35]. Note phases of the acquisition and recall sessions that contained blue or red CS+ were analyzed separately. To reduce between-subjects variability created by individual differences in startle magnitude overall, each individual participant's blocks were standardized into Z-scores such that all scores represented departure from each individual's mean level of potentiated startle across the entire experiment. Fear extinction recall was quantified as the initial fear response to

the blue CS+ (first 4 recall session CS+ Z-scores) averaged together during the extinction recall session on day 3.

Polysomnography

Polysomnography (PSG) recordings were acquired using a Grass Heritage Gamma Digital PSG system with gold cup electrodes, in the J Christian Gillin Sleep Laboratory at the VA San Diego Healthcare System. The recording montage consisted of six electroencephalogram (EEG) channels (electrode positions F3, F4, C3, C4, O1, O2) referenced to contralateral mastoids (M1, M2), 2 electrooculogram (EOG) channels, and 2 submental EMG channels. Signals were recorded at 200 Hz, and subsequently exported with a 0.3-35 Hz band pass filter (plus 60 Hz notch filter) for sleep scoring. All nights of sleep were scored in accordance with American Academy of Sleep Medicine guidelines [36].

Power spectral density

First, artifacts in the PSG were removed with an automated algorithm. Using frontal and central channels we calculated per-epoch summary metrics of three Hjorth parameters (signal activity, mobility, and complexity;). Epochs where at least 1 channel was > 3 standard deviations from the mean on any of the three Hjorth parameters were marked as artifacts and removed from subsequent analysis [37]. Artifact detection was performed over two iterations, and performed separately for each sleep stage. Power spectral density (PSD) was estimated at all six EEG electrodes, for all artifact free sleep separately for NREM (N2+N3) and REM, using Welch's method with 5s windows and 50% overlap. Primary statistical analyses were conducted on the average of the two central electrodes. Our choice of focusing on the central electrodes was primarily driven by our previous report where we found spectral power differences in PTSD at central electrode sites [28]. Estimates were obtained from the 1st derivative of the EEG time series to minimize 1/f scaling [38]. Given that signal amplitude is at least partly driven by individual difference factors such as skull thickness and gyral folding [39], we then normalized, within participant, each electrode's power spectrum by dividing power at each frequency by that electrode's average power [28,40].

Statistical analysis

Statistical analysis of PSD estimates were primarily performed using cluster-based permutation testing, implemented in the FieldTrip toolbox for MATLAB [41]. This approach allowed us to take the full power spectrum into account (and thus preserve its continuous nature), while simultaneously controlling for multiple comparisons. In other words, we were able to test our predictions about both theta and beta power within a single analysis, controlling for multiple comparisons across frequencies. Such an analysis also allowed us to detect effects in other frequency bands for which we had no *a priori* hypotheses, again without inflating the number of comparisons.

To test our primary research question, we assessed the presence of correlations between 0-30Hz PSD during sleep on the *pre-extinction* night (hereafter referred to as Night 1; **Figure 1A**) and fear extinction recall on Day 3. This analysis included participants from the Normal sleep and Post-extinction sleep deprivation groups (**Figure 1A**). The analysis was performed using the *ft_statfun_correlationT* FieldTrip function with the following parameters: 10,000 interactions, a *clusteralpha* of .05 with the default

maxsum method to determine cluster significance, and a significance threshold of .05. Separate tests were performed for REM and NREM sleep. To better visualize significant correlations, scatterplots were also created. For these, PSD at each frequency that formed part of a significant cluster was averaged together to provide a single value for the purpose of visualization in the scatterplot. We report on PSD estimates from averaged central channels, though we note that primary results were largely unchanged when using PSD estimates from averaged frontal channels.

To test our secondary research question, we ran the same correlation analysis as above, but utilizing PSD estimates during sleep on the *post-extinction* night (hereafter referred to as Night 2; **Figure 1A**). This analysis included participants from the Normal sleep and the Pre-extinction sleep deprivation groups (**Figure 1A**). Between-group differences in the magnitudes of association were compared using the Fischer r-to-z transform, and within-group differences between nights were compared using Meng's z test. An important caveat of this analysis is that participants in the Pre-extinction deprivation group were undergoing a night of recovery sleep following total sleep deprivation the night before, and therefore may exhibit significantly altered sleep microarchitecture. Slow wave activity (SWA; 0.5-4Hz PSD) has been found to be a reliable marker of homeostatic sleep pressure and is elevated in recovery sleep following sleep deprivation [42]. Therefore, we explored the within-subject change in SWA between change for the two groups.

Results

Group differences in extinction recall in the healthy control sample have been previously reported elsewhere (Straus et al, 2017), but we re-summarize them here (**Figure 1B**). Extinction recall was significantly impaired in the Pre-extinction sleep deprivation group compared to the Normal sleep group (t (44) = -2.15, p = .037, d = 0.65). The Post-extinction sleep deprivation group did not differ in extinction recall compared to the Normal sleep group (t (44) = -0.53, p = .59, d = 0.16).

Night 1

We next turned to the relationship between sleep spectral power and extinction recall. We first examined sleep PSD on Night 1, i.e. sleep following fear acquisition and prior to extinction learning, and its association with subsequent recall of extinguished fear. During REM sleep, a significant correlation cluster emerged for beta band frequencies, ranging from 19.34 - 25.97 Hz (t_{sum} = 92.63, p = .014; **Figure 2A**). This translated into a cluster-averaged correlation r = .41, p = .004 (**Figure 2B**), indicating that greater beta PSD during REM sleep was associated with worse recall of extinguished fear. The magnitude of the correlation was not significantly different between the Normal sleep group (r = .35) and the Post-extinction sleep deprivation group (r = .48), z = 0.51, p = .61. No other significant clusters emerged, indicating there was no association between theta band frequencies and fear extinction retention.

With regards to NREM sleep, a near identical pattern emerged. A significant correlation cluster was found for frequencies in the beta band (16.99 - 23.63 Hz; t_{sum} = 101.19, p = .010), indicating that greater beta PSD during NREM sleep was also associated with worse recall of extinguished fear (**Figure 2C**). The cluster-averaged correlation magnitude was identical to REM sleep (r = .41, p = .004; **Figure 2D**), with no

differences in correlation magnitude between the Normal sleep (r = .35) and Post-extinction sleep deprivation group (r = .48), z = 0.51, p = .61).

Night 2

We next examined PSD on Night 2, i.e. sleep following extinction learning and prior to extinction recall. No significant clusters emerged for either REM or NREM sleep, suggesting no relationship between sleep spectral power on Night 2 and extinction retention. This could be because post-extinction learning sleep is not predictive of extinction recall or could be because one of the groups in this analysis was recovering from a night of sleep deprivation. When we examined slow wave activity SWA significantly increased between baseline and night 2 for the Pre-extinction deprivation group, who were recovering from sleep deprivation (t (24) = 3.56, p = .002). No change in SWA was observed in the Normal sleep group (t (20) = 0.45, p = .65). A comparison of Night 2 spectral power between the two groups yielded no significant clusters for either REM (**Figure 3A**) or NREM (**Figure 3B**), though we note low statistical power for this between-subjects analysis. When we restricted analysis to just the Normal sleep group, the correlation between beta-band PSD (collapsing across frequencies which were significant on Night 1) on Night 2 extinction recall was of similar magnitude to Night 1 (REM: r = .42; NREM: r = .34). For both sleep stages, the difference in correlation magnitude was not significant (REM: z = 1.02, p = .31; NREM: z = 0.13, p = .90).

In the Pre-extinction deprivation group, the correlation between Night 2 beta power and extinction recall was not statistically significant, showing either no (REM: r = .09, p = .67; **Figure 3C**) or a non-significant negative relationship (NREM: r = -.30, p = .15; **Figure 3D**). For NREM sleep, there was a significant difference in correlation magnitude between the two groups (z = 2.09, p = .037). The REM sleep correlation magnitudes were not significantly different from each other (z = 1.02, p = .31)

Stability of beta PSD across nights

Finally, we examined whether beta PSD estimates were stable across nights. Using the Normal sleep group, we were able to investigate the stability of sleep beta activity across the three experimental nights (Baseline, Night 1, Night 2; both sleep deprivation groups were excluded from this analysis). There was no significant difference in beta power between the three nights for either REM (F (2, 40) = 1.88, p = .166, np^2 = .086) or NREM (F (2, 40) = 0.68, p = .512, np^2 = .033) sleep (**Figure 4A-B**). Next, intraclass correlation coefficients (ICC) were used to quantify the level of similarity of an individual's beta power across the three nights. ICCs were found to be very high (REM ICC = 0.94 [0.89, 0.97]; NREM ICC = 0.93 [95% CI: 0.87, 0.97]), indicating a high agreement in beta PSD across nights (see **Figure 4C-D** for illustrative examples).

Interim summary

These results suggest that in healthy participants, heightened beta power during both NREM and REM sleep were associated with impaired recall of extinguished fear. As such, high beta power during sleep exert a similar negative effect on extinction recall as a night of sleep deprivation. Beta PSD estimates were highly consistent across nights, suggesting them to be a stable marker of an individual's sleep microarchitecture.

Study 2

Methods

This study was a reanalysis of a previously published dataset [30] of individuals with PTSD. Please see [30] for full details. The investigation was carried out in accordance with the Declaration of Helsinki. The VA Internal Review Board as well as the University of California, San Diego's Human Research Protections Program approved the study. Informed consent was obtained from all participants.

Participants

Study 2 participants were 15 Veterans with a primary diagnosis of PTSD. A total of 12 participants with complete, usable data were included (M_{age} = 29 years, SD_{age} = 5 years; 2 female). Participants had no history of psychosis, substance use disorders in the six months prior to the study, and no untreated sleep disorders other than insomnia and nightmares. Included participants exhibited a consistent startle response at screening (over 75% discernable responses to twelve 105dB 40ms startle pulses).

Procedure

Participants followed the same procedure as the Normal sleep group of Study 1 (**Figure 1A**), except the baseline night also served as an adaptation night. PSG recordings were identical that of Study 1.

Fear potentiated startle

Fear acquisition started with the same six trial acclimation period as Study 1. Following acclimation, participants were exposed to: 1) eight 6-second long presentations of a blue circle (CS+), paired with an air puff (US) to the throat in 75% of trials; 2) eight 6-second long presentations of a yellow circle that was never paired with an air puff (CS-); and 3) 8 presentations of the startle pulse in the absence of any stimuli. Stimulus presentation was block randomized with the constraint of two trials of each type per block. On day 2, participants underwent extinction learning, consisting of 16 presentations each of CS+, CS-, blank screen along with startle pulses but without any air puffs being delivered. On day 3, participants performed the extinction recall session. This session followed the exact same procedure as initial fear acquisition, except no air puffs were delivered.

Statistical analysis

Analysis of extinction recall and spectral analysis were identical to Study 1. To test for group differences, we compared Night 1 spectral power between Healthy controls (Normal sleep and Post-extinction deprivation groups only) and individuals with PTSD, using the same cluster-based permutation framework as in study 1, with the *ft_statfun_indepsamplesT* function to compare the two groups. We focused our group difference analysis on Night 1 rather than the Baseline night because Baseline night also served as adaptation night for the PTSD group (**Figure 1A**), meaning that any group differences could have been due to a first-night effect. Equally, by performing our group difference analysis on Night 2, our healthy control sample would have contained participants who were recovering from a night of sleep deprivation, and removing this group would greatly reduce our statistical power. To obtain an

effect size for each significant cluster, PSD values for each frequency within a given cluster were averaged together, and the HC vs PTSD group difference was compared with Cohen's *d*.

We examined the relationship between sleep spectral power and extinction recall in PTSD by correlating PSD averaged across frequencies which revealed a significant group difference in the above analysis. Because our analysis of healthy controls found associations with beta band power only, in order to limit the number of comparisons, we focused our correlational analysis only on group-differences that emerged in the canonical beta band (~16 - 30Hz).

Results

Group differences

"The PTSD sample did not differ significantly in terms of extinction recall from the normal sleep group (t (24.6) = 0.88, p = .39, d = 0.31), and showed significantly better extinction recall compared to the pre-extinction sleep deprivation group (t (34.1) = 2.80, p = .008, d = 0.89; **Figure 1B**), though we note the differences in FPS procedure between the healthy control and patient study (see Methods)."

Sleep statistics averaged across the three nights are displayed in **Table 1**. See **Table S1** for each group and night separately. Compared to healthy participants, those with PTSD had lower total sleep time (*t* (62.1) = 6.97, *p* < .001, *d* = 1.46). As a percentage of total sleep time, PTSD participants showed reduced REM time (*t* (72.6) = 2.28, *p* = .026, *d* = 0.47) and a corresponding increase in N2 time (*t* (62.6) = 2.31, *p* = .024, *d* = 0.48).

During REM sleep, we found significantly higher PSD in the beta frequency range (17.58-23.83Hz; t_{sum} = 105.15, *p* = .007, *d* = 0.90; **Figure 5A**) in PTSD relative to controls. We additionally observed significantly lower high theta/low alpha REM PSD in PTSD (7.03-10.94Hz; t_{sum} = 68.03, *p* = .030, d = 1.22). When we assessed group differences in NREM sleep, we again found significantly higher beta PSD in PTSD relative to controls (15.82-21.48Hz; t_{sum} = 81.13, *p* = .038, *d* = 0.62; **Figure 5B**).

Correlations with extinction recall

We correlated cluster-averaged Night 1 beta power (i.e. beta-band frequencies which significantly differed between the groups) with fear extinction recall. We did not observe a correlation between beta power during REM sleep and extinction recall in the PTSD sample (*r* = .12 *p* = .704; Healthy controls: *r* = .37, *p* = .011; **Figure 5C**). For the NREM cluster however, a non-significant positive association was observed (PTSD: *r* = .39, *p* = .214; Healthy controls: *r* = .37, *p* = .011; **Figure 5D**). The lack of statistical significance in the PTSD group may be attributed to the small sample size. Similar patterns in the PTSD group emerged when we ran the analysis on Night 2 PSD estimates (REM: *r* = .28, *p* = .40; NREM: *r* = .42, *p* = .20).

Stability of beta PSD estimates in PTSD

Finally, we examined whether PSD estimates in the frequency band associated with impaired extinction recall is also a stable measure in PTSD. As with healthy controls, beta spectral power was found to be highly stable across all three nights in PTSD (REM: ICC = .94 [.87, .97]; NREM: ICC = .92 [95%CI: .78, .98]).

In summary, this provides the first, albeit highly preliminary, evidence that beta frequency activity during sleep, characteristic of PTSD sleep, is indeed associated with the often-documented deficit in fear extinction recall seen in this group.

Discussion

We found that increased beta spectral power during pre-extinction learning sleep (Night 1) was associated with poorer extinction recall. This association was significant for both NREM and REM sleep. This study is, to our knowledge, the first study to associate beta-band spectral power with fear extinction recall processes. Beta activity is often considered a marker of cortical hyperarousal and otherwise disturbed sleep. Our results show that these sleep disturbances are linked to a reduced ability to recall extinguished fear. These results are important considering that higher levels of beta power have been reported in both PTSD and insomnia [20,43]. This mirrors that of prior work using sleep deprivation, which found an impairment in extinction recall when total sleep deprivation preceded, rather than followed, initial extinction learning [15].

Pre-encoding sleep loss has been linked to attenuated functioning in memory-related brain structures, such as the hippocampus [44–46], as well as impaired consolidation, even following subsequent periods of recovery sleep [46]. In a fear conditioning task, neuroimaging work has shown a failure to activate both fear and extinction processing-related brain regions following either sleep restriction or total sleep deprivation [47]. Similarly, beta power during sleep has also been suggested as a proxy of disturbed sleep, and is related to increased autonomic arousal and the disturbance of both NREM and REM sleep [31]. As such, it is possible that sleep disturbance, indexed here as heightened beta spectral power, led to changes in the neural substrates of extinction learning, which in turn led to poor consolidation of the fear extinction process.

A notable finding was that Night 1 beta spectral power predicted extinction recall in the post-extinction deprivation group, who went on to be sleep deprived the following night (Night 2). In the Normal sleep group, the relationship between beta spectral power and extinction retention was equivalent in magnitude on both Night 1 and Night 2, suggesting that beta spectral power levels consistently predict extinction recall ability, independently of when sleep occurs relative to extinction learning. This result is consistent with our finding that beta PSD estimates were highly consistent between nights.

The high night-to-night consistency of beta PSD in both healthy controls and PTSD aligns with a large body of work showing the sleep EEG to be a highly stable and heritable trait [37,48–50]. Higher beta power during sleep (relative to healthy controls) has been documented in a number of psychopathologies, including PTSD [18,19,21]. Notably, when individuals with PTSD are compared to trauma-exposed, non-PTSD participants, higher beta power is not always observed [28,29,51]. Prospective, longitudinal research in this area may be able to identify certain sleep oscillatory activity as a characteristic biomarker with regards to the risk or resilience toward the onset of PTSD following a trauma exposure.

As well as finding beta spectral power to be heightened in PTSD relative to healthy controls, we also found that the association between pre-extinction learning NREM beta power was associated with poorer extinction memory at a magnitude similar to healthy controls, however we stress that the

correlation was not statistically significant. We therefore speculate that beta spectral power may be a stable sleep biomarker that confers vulnerability to impaired consolidation of fear extinction. While this result is both novel and intriguing, we stress that these results should be considered preliminary due to the small sample size, and so must be replicated in larger samples. While the relationship between NREM beta power and extinction recall showed a similar strength of association in both healthy participants and PTSD, this was not the case for REM sleep, where the correlation was smaller in PTSD. This could reflect a functional dissociation in NREM vs REM sleep microarchitecture following a trauma, especially given that numerous alterations in REM architecture and physiology have been observed in PTSD [4]. Furthermore, work in other clinical groups such as insomnia has observed opposing relationships between REM sleep and fear extinction recall between patients and controls [8].

We found novel evidence that beta band spectral power during sleep is negatively associated with fear extinction recall. However, the mechanistic basis and origins of the beta rhythm in the sleep EEG remains poorly understood. Beta activity is often cited as a marker of cortical hyperarousal, and has frequently been shown to be heightened in insomnia [43]. Such a link is not always found however. For example, following a mindfulness-based intervention for chronic insomnia, NREM beta power increased from baseline and was associated with fewer symptoms [52]. In a large sample of PTSD patients and trauma-exposed controls, beta power levels were associated with fewer symptoms of subjective hyperarousal, fewer nightmares, and improved emotion regulation [28] As such, it has been proposed that while beta activity during sleep may index heightened arousal, the nature of this hyperarousal may either be adaptive or maladaptive [52].

While we found that beta band activity was associated with poorer extinction recall, we did not find any evidence of an association between REM theta (~4-8Hz) PSD and extinction recall. Some studies have found a link between emotional memory recollection and REM theta power [53,54], especially when emotional memories are encoded under stressful conditions [55]. However, this link is not consistent across the existing literature [27], and enhancing REM theta oscillations through acoustic stimulation does not lead to expected improvements in emotional memory [56]. Some work has also suggested that the magnitude of REM theta oscillations may be predictive of resilience to developing PTSD after trauma exposure. The current data however do not support a role of REM theta power in extinction retention processes. As such, future work should explore other mechanisms by which REM theta may be protective against PTSD development.

The present study has several limitations that must be acknowledged. First, there were slight differences in the fear conditioning protocol between Study 1 (Healthy controls) and Study 2 (PTSD). In Study 1, the unconditioned stimulus consisted of an electric shock, whereas in Study 2 consisted of an air puff. Although both approaches are widely used in the fear conditioning literature, this methodological difference may account for why, at a behavioral level, extinction recall was no worse in PTSD compared to controls. Although this difference is a limitation, we note that our primary goal here was not to compare group differences in extinction recall itself, but rather determine associations between sleep spectral power and the ability to recall extinguished fear. The fact that, for NREM sleep at least, correlations were of a similar magnitude in both groups (although not significantly in the PTSD sample) suggests similar sleep mechanisms contributed to extinction recall processes in the two studies. Future work, using primary datasets where methodologies are equivalent across all groups, should aim to replicate these findings. Another limitation is that the association between beta power and extinction

recall is purely correlational, and as such causal inferences cannot be conclusively made from this data alone. Acoustic and electrical stimulation methods can be used to enhance frequency-specific sleep oscillations that have been shown to either promote [57] or impair [58] memory consolidation, and are a fruitful target for future research. We also acknowledge that the PTSD dataset was a pilot investigation and contained a sample too small to reliably detect significant correlations at the magnitude found in the healthy controls. We did not collect any measures of insomnia symptom severity, meaning we were unable to investigate whether beta spectral power was linked to any symptoms of hyperarousal seen in insomnia. Finally, the inclusion of a trauma-exposed, non-PTSD control group would help to refine whether our findings reflect alterations in PTSD specifically, or trauma exposure more generally.

Conclusion

Sleep disturbances are extremely common in PTSD, and contribute to its onset and maintenance. We found evidence that beta spectral power was associated with subsequent ability to recall extinguished fear in healthy controls, with a non-significant trend in the same direction in PTSD. Given the importance of extinction recall for the treatment of PTSD symptoms, this work provides an important new contribution to our understanding of the electrophysiological underpinnings of a key mechanism of PTSD maintenance.

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Disclosure statement

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Non-financial disclosure: none

Data Availability Statement

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

References

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Health Disorders. 5th ed. Washington, DC: American Psychiatric Association; 2013.
2. Colvonen PJ, Straus LD, Acheson D, Gehrman P. A Review of the Relationship Between Emotional Learning and Memory, Sleep, and PTSD. Curr Psychiatry Rep. 2019;21:2.
3. Milad MR, Quirk GJ. Fear extinction as a model for translational neuroscience: ten years of progress. Annu Rev Psychol. 2012;63:129–51.
4. Pace-Schott EF, Germain A, Milad MR. Effects of sleep on memory for conditioned fear and fear extinction. Psychological Bulletin. 2015;141:835–57.
5. Foa E, Hembree EA, Rothbaum BO, Rauch S. Prolonged Exposure Therapy for PTSD: Emotional Processing of Traumatic Experiences - Therapist Guide [Internet]. Oxford University Press; 2019 [cited 2022 Feb 9]. Available from: https://doi.org/10.1093/med-psych/9780190926939.001.0001
6. Germain A. Sleep Disturbances as the Hallmark of PTSD: Where Are We Now? AJP. 2013;170:372–82.
7. Zhang Y, Ren R, Sanford LD, Yang L, Zhou J, Zhang J, et al. Sleep in posttraumatic stress disorder: A systematic review and meta-analysis of polysomnographic findings. Sleep Medicine Reviews. 2019;48:101210.
8. Bottary R, Seo J, Daffre C, Gazecki S, Moore KN, Kopotiyenko K, et al. Fear extinction memory is negatively associated with REM sleep in insomnia disorder. Sleep [Internet]. 2020 [cited 2020 Jul 28];43. Available from: http://academic.oup.com/sleep/article/43/7/zsaa007/5717136
9. Hauner KK, Howard JD, Zelano C, Gottfried JA. Stimulus-specific enhancement of fear extinction during slow-wave sleep. Nat Neurosci. 2013;16:1553–5.
10. He J, Sun H-Q, Li S-X, Zhang W-H, Shi J, Ai S-Z, et al. Effect of conditioned stimulus exposure during slow wave sleep on fear memory extinction in humans. Sleep. 2015;38:423–31.
11. Pace-Schott EF, Tracy LE, Rubin Z, Mollica AG, Ellenbogen JM, Bianchi MT, et al. Interactions of time of day and sleep with between-session habituation and extinction memory in young adult males. Exp Brain Res. 2014;232:1443–58.
12. Schenker MT, Ney LJ, Miller LN, Felmingham KL, Nicholas CL, Jordan AS. Sleep and fear conditioning, extinction learning and extinction recall: A systematic review and meta-analysis of polysomnographic findings. Sleep Medicine Reviews. 2021;59:101501.
13. Spoormaker VI, Sturm A, Andrade KC, Schröter MS, Goya-Maldonado R, Holsboer F, et al. The neural correlates and temporal sequence of the relationship between shock exposure, disturbed sleep and impaired consolidation of fear extinction. J Psychiatr Res. 2010;44:1121–8.
14. Spoormaker VI, Schröter MS, Andrade KC, Dresler M, Kiem SA, Goya-Maldonado R, et al. Effects of rapid eye movement sleep deprivation on fear extinction recall and prediction error signaling. Hum Brain Mapp. 2012;33:2362–76.
15. Straus LD, Acheson DT, Risbrough VB, Drummond SPA. Sleep Deprivation Disrupts Recall of Conditioned Fear Extinction. Biological Psychiatry: Cognitive Neuroscience and Neuroimaging. 2017;2:123–9.
16. Schäfer SK, Wirth BE, Staginnus M, Becker N, Michael T, Sopp MR. Sleep's impact on emotional recognition memory: A meta-analysis of whole-night, nap, and REM sleep effects. Sleep Medicine Reviews. 2020;101280.
17. Cowdin N, Kobayashi I, Mellman TA. Theta frequency activity during rapid eye movement (REM) sleep is greater in people with resilience versus PTSD. Exp Brain Res. 2014;232:1479–85.
18. de Boer M, Nijdam MJ, Jongedijk RA, Bangel KA, Olff M, Hofman WF, et al. The spectral fingerprint of sleep problems in post-traumatic stress disorder. Sleep. 2020;43:zsz269.
19. Germain A, Hall M, Shear MK, Nofzinger EA, Buysse DJ. Ecological Study of Sleep Disruption in PTSD. Annals of the New York Academy of Sciences. 2006;1071:438–41.

20. Wang C, Ramakrishnan S, Laxminarayan S, Dovzhenok A, Cashmere JD, Germain A, et al. An attempt to identify reproducible high-density EEG markers of PTSD during sleep. Sleep [Internet]. 2020 [cited 2021 Apr 14];43. Available from: https://doi.org/10.1093/sleep/zsz207
21. Woodward SH, Murburg MM, Bliwise DL. PTSD-related hyperarousal assessed during sleep. Physiology & Behavior. 2000;70:197–203.
22. Hall M, Thayer JF, Germain A, Moul D, Vasko R, Puhl M, et al. Psychological Stress Is Associated With Heightened Physiological Arousal During NREM Sleep in Primary Insomnia. Behavioral Sleep Medicine. 2007;5:178–93.
23. Perlis ML, Smith MT, Andrews PJ, Orff H, Giles DE. Beta/Gamma EEG Activity in Patients with Primary and Secondary Insomnia and Good Sleeper Controls. Sleep. 2001;24:110–7.
24. Riedner BA, Goldstein MR, Plante DT, Rumble ME, Ferrarelli F, Tononi G, et al. Regional Patterns of Elevated Alpha and High-Frequency Electroencephalographic Activity during Nonrapid Eye Movement Sleep in Chronic Insomnia: A Pilot Study. Sleep. 2016;39:801–12.
25. Spiegelhalder K, Regen W, Feige B, Holz J, Piosczyk H, Baglioni C, et al. Increased EEG sigma and beta power during NREM sleep in primary insomnia. Biological Psychology. 2012;91:329–33.
26. Richards A, Metzler TJ, Ruoff LM, Inslicht SS, Rao M, Talbot LS, et al. Sex differences in objective measures of sleep in post-traumatic stress disorder and healthy control subjects. Journal of Sleep Research. 2013;22:679–87.
27. Davidson P, Jönsson P, Carlsson I, Pace-Schott E. Does Sleep Selectively Strengthen Certain Memories Over Others Based on Emotion and Perceived Future Relevance? NSS. 2021;13:1257–306.
28. Denis D, Bottary R, Cunningham TJ, Zeng S, Daffre C, Oliver KL, et al. Sleep Power Spectral Density and Spindles in PTSD and Their Relationship to Symptom Severity. Frontiers in Psychiatry. 2021;12:2070.
29. Mellman TA, Pigeon WR, Nowell PD, Nolan B. Relationships between REM sleep findings and PTSD symptoms during the early aftermath of trauma. Journal of Traumatic Stress. 2007;20:893–901.
30. Straus LD, Norman SB, Risbrough VB, Acheson DT, Drummond SPA. REM sleep and safety signal learning in posttraumatic stress disorder: A preliminary study in military veterans. Neurobiology of Stress. 2018;9:22–8.
31. Kuo TBJ, Chen C-Y, Hsu Y-C, Yang CCH. EEG beta power and heart rate variability describe the association between cortical and autonomic arousals across sleep. Auton Neurosci. 2016;194:32–7.
32. Marshall AJ, Acheson DT, Risbrough VB, Straus LD, Drummond SPA. Fear Conditioning, Safety Learning, and Sleep in Humans. J Neurosci. 2014;34:11754–60.
33. Lonsdorf TB, Menz MM, Andreatta M, Fullana MA, Golkar A, Haaker J, et al. Don't fear 'fear conditioning': Methodological considerations for the design and analysis of studies on human fear acquisition, extinction, and return of fear. Neuroscience & Biobehavioral Reviews. 2017;77:247–85.
34. Acheson D, Feifel D, de Wilde S, Mckinney R, Lohr J, Risbrough V. The effect of intranasal oxytocin treatment on conditioned fear extinction and recall in a healthy human sample. Psychopharmacology. 2013;229:199–208.
35. Milad MR, Wright CI, Orr SP, Pitman RK, Quirk GJ, Rauch SL. Recall of Fear Extinction in Humans Activates the Ventromedial Prefrontal Cortex and Hippocampus in Concert. Biological Psychiatry. 2007;62:446–54.
36. Iber C, Ancoli-Israel S, Chesson A, Quan SF. The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specification. Westchester, IL: Americian Academy of Sleep Medicine; 2007.
37. Purcell SM, Manoach DS, Demanuele C, Cade BE, Mariani S, Cox R, et al. Characterizing sleep spindles in 11,630 individuals from the National Sleep Research Resource. Nat Commun. 2017;8:15930.
38. Cox R, Schapiro AC, Manoach DS, Stickgold R. Individual differences in frequency and topography of slow and fast sleep spindles. Front Hum Neurosci. 2017;11:433.
39. Cox R, Fell J. Analyzing human sleep EEG: A methodological primer with code implementation. Sleep Medicine Reviews. 2020;54:101353.

40. Cunningham TJ, Bottary R, Denis D, Payne JD. Sleep spectral power correlates of prospective memory maintenance. Learn Mem. 2021;28:291–9.
41. Maris E, Oostenveld R. Nonparametric statistical testing of EEG- and MEG-data. J Neurosci Methods. 2007;164:177–90.
42. Riedner BA, Vyazovskiy VV, Huber R, Massimini M, Esser S, Murphy M, et al. Sleep Homeostasis and Cortical Synchronization: III. A High-Density EEG Study of Sleep Slow Waves in Humans. Sleep. 2007;30:1643–57.
43. Zhao W, Van Someren EJW, Li C, Chen X, Gui W, Tian Y, et al. EEG spectral analysis in insomnia disorder: A systematic review and meta-analysis. Sleep Medicine Reviews. 2021;59:101457.
44. Poh J-H, Chee MWL. Degradation of neural representations in higher visual cortex by sleep deprivation. Sci Rep. 2017;7:45532.
45. Prince T-M, Abel T. The impact of sleep loss on hippocampal function. Learn Mem. 2013;20:558–69.
46. Yoo S-S, Gujar N, Hu P, Jolesz FA, Walker MP. The human emotional brain without sleep — a prefrontal amygdala disconnect. Current Biology. 2007;17:R877–8.
47. Seo J, Pace-Schott EF, Milad MR, Song H, Germain A. Partial and Total Sleep Deprivation Interferes With Neural Correlates of Consolidation of Fear Extinction Memory. Biological Psychiatry: Cognitive Neuroscience and Neuroimaging. 2021;6:299–309.
48. Ambrosius U, Lietzenmaier S, Wehrle R, Wichniak A, Kalus S, Winkelmann J, et al. Heritability of sleep electroencephalogram. Biol Psychiatry. 2008;64:344–8.
49. Buckelmüller J, Landolt H-P, Stassen HH, Achermann P. Trait-like individual differences in the human sleep electroencephalogram. Neuroscience. 2006;138:351–6.
50. De Gennaro L, Ferrara M, Vecchio F, Curcio G, Bertini M. An electroencephalographic fingerprint of human sleep. NeuroImage. 2005;26:114–22.
51. Cohen DJ, Begley A, Alman JJ, Cashmere DJ, Pietrone RN, Seres RJ, et al. Quantitative electroencephalography during rapid eye movement (REM) and non-REM sleep in combat-exposed veterans with and without post-traumatic stress disorder. Journal of Sleep Research. 2013;22:76–82.
52. Goldstein MR, Turner AD, Dawson SC, Segal ZV, Shapiro SL, Wyatt JK, et al. Increased high-frequency NREM EEG power associated with mindfulness-based interventions for chronic insomnia: Preliminary findings from spectral analysis. Journal of Psychosomatic Research. 2019;120:12–9.
53. Nishida M, Pearsall J, Buckner RL, Walker MP. REM sleep, prefrontal theta, and the consolidation of human emotional memory. Cereb Cortex. 2009;19:1158–66.
54. Sopp MR, Michael T, Weeß H-G, Mecklinger A. Remembering specific features of emotional events across time: The role of REM sleep and prefrontal theta oscillations. Cogn Affect Behav Neurosci. 2017;17:1186–209.
55. Kim SY, Kark SM, Daley RT, Alger SE, Rebouças D, Kensinger EA, et al. Interactive effects of stress reactivity and rapid eye movement sleep theta activity on emotional memory formation. Hippocampus. 2020;30:829–41.
56. Harrington MO, Ashton JE, Ngo H-VV, Cairney SA. Phase-locked auditory stimulation of theta oscillations during rapid eye movement sleep. Sleep. 2020;44:zsaa227.
57. Wunderlin M, Züst MA, Hertenstein E, Fehér KD, Schneider CL, Klöppel S, et al. Modulating overnight memory consolidation by acoustic stimulation during slow-wave sleep: a systematic review and meta-analysis. Sleep. 2021;44:zsaa296.
58. Marshall L, Kirov R, Brade J, Mölle M, Born J. Transcranial Electrical Currents to Probe EEG Brain Rhythms and Memory Consolidation during Sleep in Humans. PLOS ONE. 2011;6:e16905.

Figure 1. Experimental design and behavioral results. **A** - Study timeline for each group. **B** - Fear extinction recall, displayed as the startle response to CS+ above baseline (Z score). Note that the PTSD sample performed a different fear potentiated startle protocol. Error bars indicate the between-subject standard error.

Figure 2. Spectral power during pre-extinction learning sleep. **A** - REM power spectrum. Frequencies showing a significant correlation with extinction recall (cluster-corrected) are highlighted in gray. Shaded area around the line indicates the between-subject standard error. **B** - Scatterplot illustrating the relationship between cluster-averaged REM PSD and fear extinction recall. **C** - NREM power spectrum. Frequencies showing a significant correlation with extinction recall (cluster-corrected) are highlighted in gray. Shaded area around the line indicates the between-subject standard error. **D** - Scatterplot illustrating the relationship between cluster-averaged NREM PSD and fear extinction recall.

Figure 3. Beta power during post-extinction learning sleep. **A** - REM power spectrum. Note that the Pre-Ext Deprivation group was recovering from a night of sleep deprivation. There were no significant group differences. Shaded area around the line indicates the between-subject standard error **B** - Scatterplot illustrating the relationship between Night 2 beta PSD and fear extinction recall (frequencies derived from the significant Night 1 cluster, see Figure 2). **C** - NREM power spectrum. There were no significant group differences. Shaded area around the line indicates the between-subject standard error. **D** - Scatterplot illustrating the relationship between Night 2 NREM beta PSD and fear extinction recall (frequencies derived from the significant Night 1 cluster), see Figure 2.

Figure 4. Beta PSD during sleep is stable across nights. **A** - Beta power during REM sleep across three nights in the normal sleep group. **B** - Beta power during NREM sleep across three nights in the normal sleep group. **C** - Correlation between REM beta power during the baseline night and during night 1 (pre-extinction learning). **D** - Correlation between NREM beta power during the baseline night and during night 1 (pre-extinction learning). Error bars indicate the within-subject standard error.

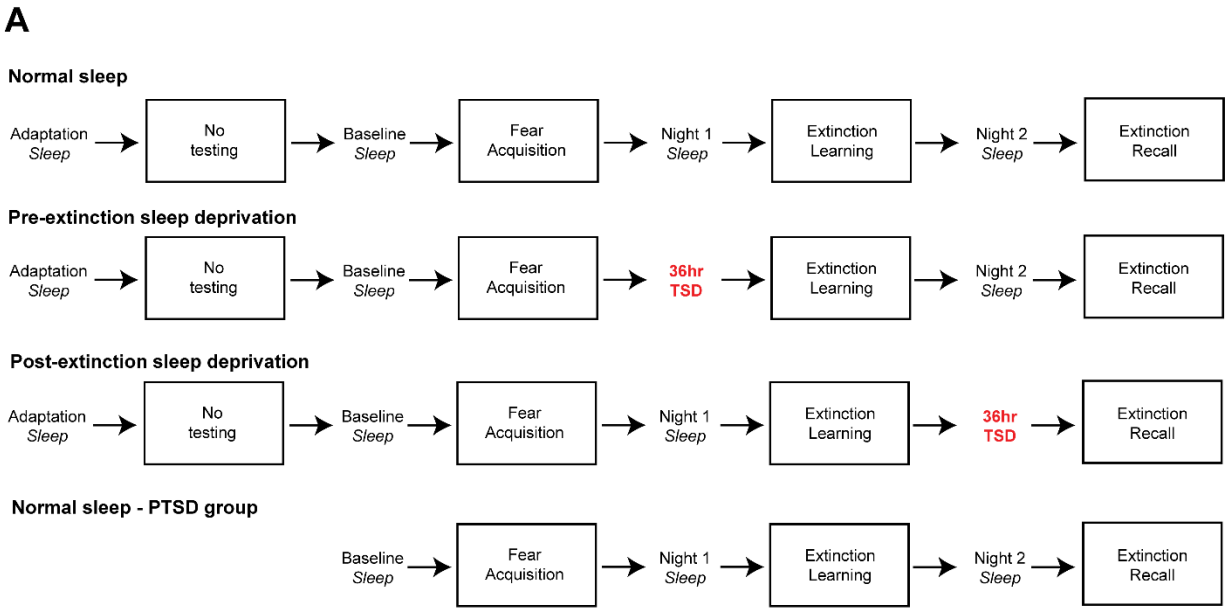
Figure 5. Spectral power differences in PTSD. **A** - REM power spectrum. Significant group differences between healthy controls and PTSD highlighted in gray. Shaded areas around the line indicate the between-subject standard error. **B** - NREM power spectrum. Significant group differences between healthy controls and PTSD highlighted in gray. Shaded areas around the line indicate the between-subject standard error. **C** - Correlation between REM beta and extinction recall in healthy controls (solid blue line) and PTSD (dashed red line). **D** - Correlation between NREM beta and

Table 1. Sleep architecture

	Healthy participants	PTSD	*t*	*p*
Total sleep time (min)	462 (38)	386 (64)	6.97	< .001
Sleep onset latency (min)	12.8 (11.2)	17.4 (23.6)	1.17	.25
Sleep efficiency (%)	91 (6.3)	90 (6.3)	0.68	.50
Wake after sleep onset (min)	34.4 (26)	29.4 (34.4)	0.80	.43
N1 (min)	38 (12.6)	27.6 (16.3)	3.50	< .001
N2 (min)	230 (34)	204 (40.5)	3.51	< .001
N3 (min)	77.7 (20.5)	66.4 (35.8)	1.84	.071
REM (min)	116.1 (22.8)	87.8 (30.1)	5.12	< .001
N1 (% of TST)	8.3 (2.8)	7.1 (4)	1.53	.13
N2 (% of TST)	49.8 (5.2)	53.2 (8.5)	2.31	.024
N3 (% of TST)	16.8 (4.4)	17.2 (9)	0.21	.24
REM (% of TST)	25.1 (4.7)	22.5 (6.2)	2.28	.026

Note. All values mean (standard deviation). Sleep architecture averaged over three experimental nights (Sleep, Night 1, Night2). Healthy participants contain the Normal sleep group only. PTSD participants slept for less time on average than healthy participants. After controlling for total sleep time, PTSD participants exhibited reduced RE and increased N2 (% of TST).

Figure 1



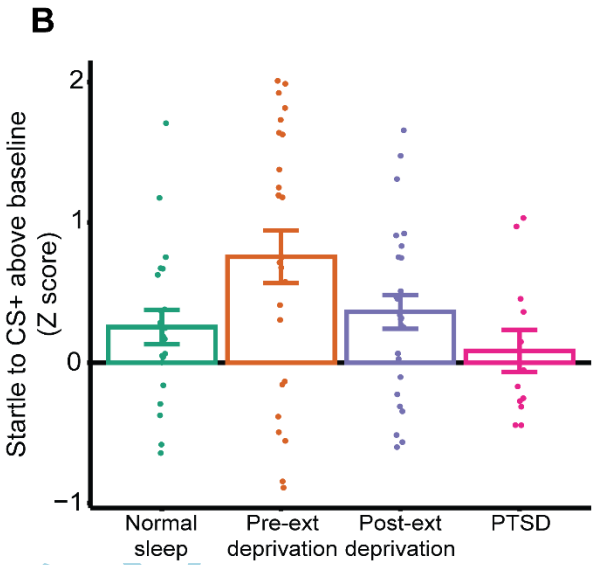


Figure 2

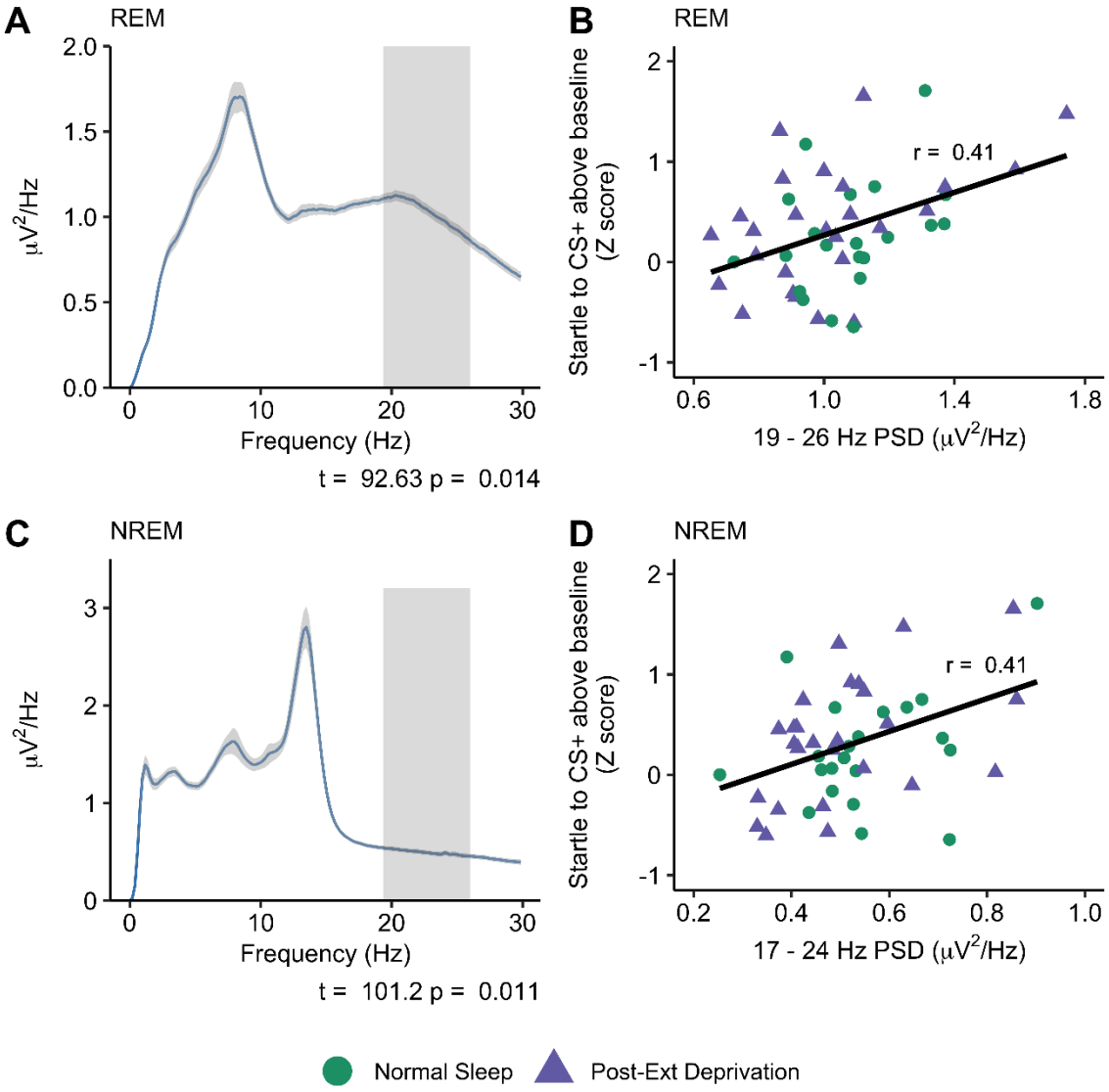


Figure 3

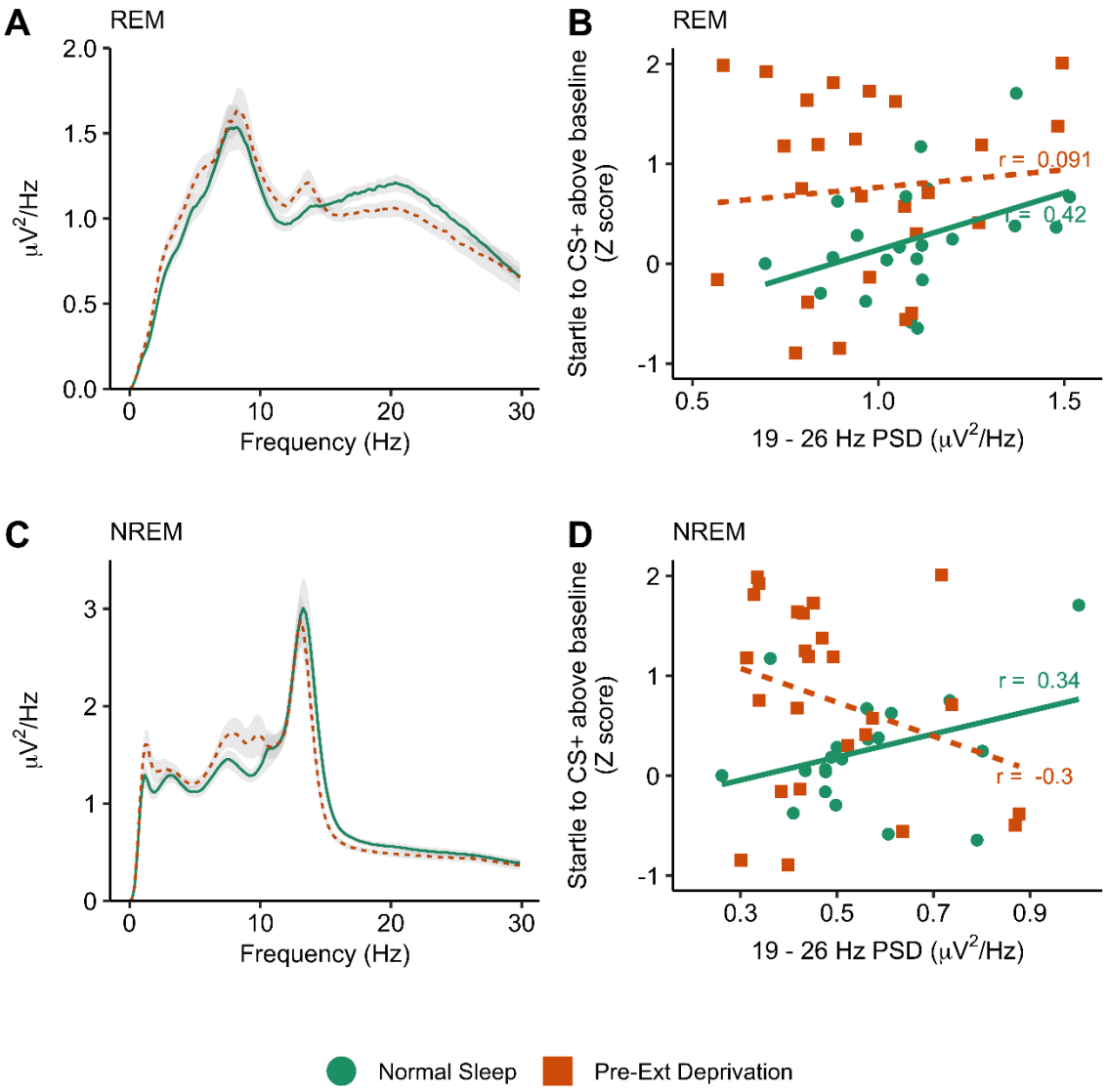


Figure 4

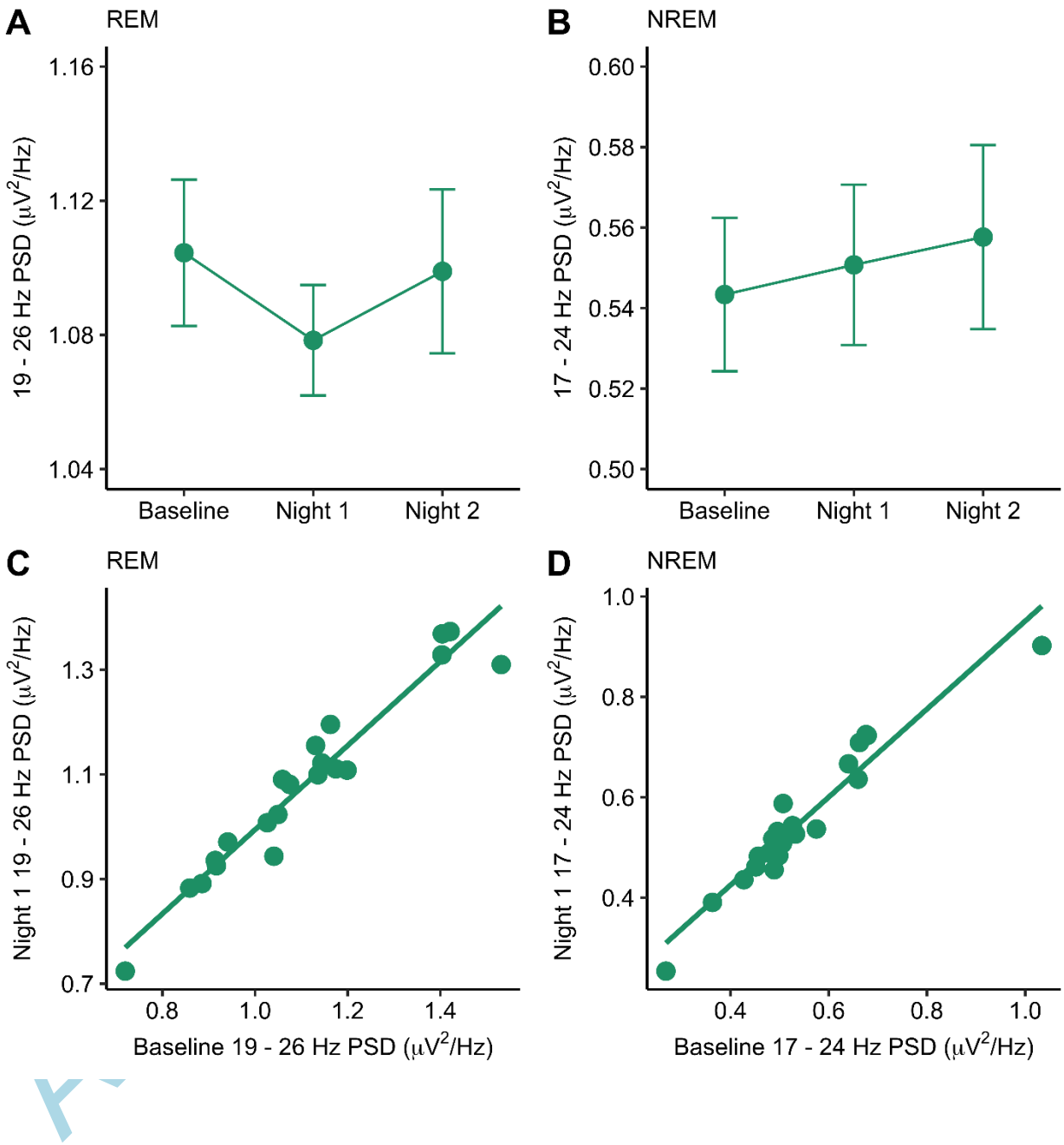


Figure 5