Original Article

Increased oscillatory frequency of sleep spindles in combat-exposed veteran men with post-traumatic stress disorder

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Abstract

Study Objectives: Sleep disturbances are core symptoms of post-traumatic stress disorder (PTSD), but reliable sleep markers of PTSD have yet to be identified. Sleep spindles are important brain waves associated with sleep protection and sleep-dependent memory consolidation. The present study tested whether sleep spindles are altered in individuals with PTSD and whether the findings are reproducible across nights and subsamples of the study.

Methods: Seventy-eight combat-exposed veteran men with (n = 31) and without (n = 47) PTSD completed two consecutive nights of high-density EEG recordings in a laboratory. We identified slow (10–13 Hz) and fast (13–16 Hz) sleep spindles during N2 and N3 sleep stages and performed topographical analyses of spindle parameters (amplitude, duration, oscillatory frequency, and density) on both nights. To assess reproducibility, we used the first 47 consecutive participants (18 with PTSD) for initial discovery and the remaining 31 participants (13 with PTSD) for replication assessment.

Results: In the discovery analysis, compared to non-PTSD participants, PTSD participants exhibited (1) higher slow-spindle oscillatory frequency over the antero-frontal regions on both nights and (2) higher fast-spindle oscillatory frequency over the centro-parietal regions on the second night. The first finding was preserved in the replication analysis. We found no significant group differences in the amplitude, duration, or density of slow or fast spindles.

Conclusions: The elevated spindle oscillatory frequency in PTSD may indicate a deficient sensory-gating mechanism responsible for preserving sleep continuity. Our findings, if independently validated, may assist in the development of sleep-focused PTSD diagnostics and interventions.

Statement of Significance

Patients with post-traumatic stress disorder (PTSD) often suffer from sleep disturbances. Sleep spindles are an electrophysiological hallmark of nonrapid eye movement sleep and are believed to be involved in sleep protection and sleep-dependent memory consolidation. This study made an initial effort to investigate whether spindle characteristics are altered in individuals with PTSD. We found that the oscillatory frequencies of sleep spindles were higher in PTSD participants than in non-PTSD participants. Importantly, the findings were consistent across nights and sub-samples of our study population. The elevated sleep-spindle frequency in PTSD may indicate a deficient sensory-gating mechanism responsible for preserving sleep continuity. Our findings provide the basis for an initial understanding of sleep-spindle abnormalities in PTSD.

Sleep disturbances, including recurrent nightmares and difficulty falling asleep or maintaining sleep, are common symptoms of post-traumatic stress disorder (PTSD). According to one study of combat-exposed veterans with PTSD, more than 50% have recurrent nightmares and over 90% have difficulty falling or staying asleep [1]. Additionally, sleep complaints before or soon after trauma exposure predict the subsequent development of PTSD [2–4], while sleep improvements in PTSD patients are accompanied by alleviation of daytime symptoms [5], suggesting that sleep disturbances are not merely secondary symptoms arising from the disorder but are instead crucial to its development and maintenance [6]. As such, studying spontaneous neural activity during sleep in PTSD may lead to the identification of objective sleep markers that indicate disease progression, assist diagnosis, and inform the development of sleep-focused interventions. However, few attempts to date have been made in this direction [7, 8] and reliable markers of neural activity during sleep in PTSD remain to be identified [9, 10].

An important neural feature of nonrapid eye movement (NREM) sleep is the occurrence of sleep spindles, which are bursts of rhythmic 10–16 Hz activity arising from the thalamocortical circuitry [11]. Functionally, sleep spindles are thought to be involved in a sensory-gating process that blocks the transmission of sensory information to the cerebral cortex and, thereby, preserve the continuity of sleep in the presence of potentially disruptive stimuli [11, 12]. In addition, sleep spindles may play a role in memory consolidation [13]. For instance, after viewing a traumatic movie, individuals with a high number of sleep spindles developed fewer intrusive trauma memories [14]. Notably, unlike conventional sleep architecture parameters (e.g. sleep efficiency) that often vary considerably from night to night [15], parameters of sleep spindles, including their amplitude (the maximum peak-to-peak difference), duration, oscillatory frequency (number of oscillatory cycles per second), and density (number of sleep-spindle events per minute), show remarkable test-retest reliability [16]. Given the properties and functional relevance of sleep spindles, and the fact that PTSD patients exhibit disrupted sleep [1] and anomalies in sleep-dependent memory processing [17], alterations in sleep-spindle parameters might serve as stable sleep markers of PTSD that reflect its underlying pathophysiology. However, to date, few if any studies have investigated this possibility. The only study that explicitly examined sleep spindles in PTSD only assessed spindle density, which was unaltered [18]. Other sleep-spindle parameters, including amplitude, duration, and oscillatory frequency, have yet to be assessed in a PTSD population.

The objective of the present study was to assess whether sleep spindles are altered in individuals with PTSD. To this end, we recorded 64-channel high-density EEG from 78 combat-exposed veteran men with (*n* = 31) and without (*n* = 47) PTSD during two consecutive nights. We detected sleep spindles using an automatic algorithm and performed topographical analyses to identify regional alterations in spindle parameters (amplitude, duration, oscillatory frequency, and density) on both nights. As accumulating evidence suggests the existence of two distinct types of sleep spindles–slow (~10–13 Hz, with predominant frontal localization) and fast (~13–16 Hz, with predominant centro-parietal localization) [19, 20]–we evaluated their parameters separately.

An important issue in previous studies to identify markers of PTSD during sleep is the lack of reproducibility. Findings from different studies have often been inconsistent [9, 10]. Therefore, to assess the reproducibility of our findings, we conducted a replication analysis within the study. Specifically, we first restricted our analyses to the first 47 consecutive participants (18 with PTSD) for initial discovery, and then examined whether we could reproduce our findings in the remaining 31 participants (13 with PTSD).

Materials and Methods

Participants

All participants provided written informed consent in accordance with the protocol approved by the University of Pittsburgh Institutional Review Board (Pittsburgh, PA) and the U.S. Army Medical Research and Development Command Human Research Protection Office (Ft. Detrick, MD).

The participants were combat-exposed veterans between the ages of 18 and 50 years, who had been deployed in support of the global war on terror. All participants were free of medications known to affect sleep or daytime functioning for at least 2 weeks. They limited their caffeine intake to no more than 2 cups of coffee (or the equivalent) per day and consumed no more than 2 alcoholic drinks per day for 2 weeks prior to visiting the sleep laboratory. The exclusion criteria also included current or untreated severe depression, substance or alcohol abuse within the past 3 months, a history of psychotic or bipolar disorder, current post-concussive symptoms or rehabilitation treatment for traumatic brain injury, a significant or unstable acute or chronic medical condition, and a current sleep disorder other than insomnia or nightmares. We did not exclude participants with a prior history of alcohol use disorder (AUD) because alcohol consumption is common in the military.

We determined the presence and severity of PTSD according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV), using the Clinician Administered PTSD Scale (CAPS) [21]. We assessed the presence, frequency, and severity of symptoms for each DSM sleep disorder using the Structured Clinical Interview for Sleep Disorders developed at the University of Pittsburgh [22]. Other clinical assessments included the Pittsburgh Sleep Quality Index (PSQI) [23] and the Insomnia Severity Index (ISI) [24] to assess sleep, the Epworth Sleepiness Scale (ESS) [25] to assess daytime sleepiness, the Patient Health Questionnaire-9 (PHQ-9) [26] to assess depression, and the Structured Clinical Interview for DSM-IV Axis I Disorders [27] to assess the presence of mood, anxiety, psychosis, alcohol use, and substance use disorders. A trained clinician administered the CAPS, performed clinical interviews, and made the final diagnosis. To assess habitual sleep patterns, we asked participants to complete a sleep diary for 10 consecutive days prior to arrival at the laboratory. We also used the diaries to monitor the daily intake of caffeine and alcohol. To rule out sleep apnea, we asked participants to wear a portable two-channel apnea screening device (ApneaLink; ResMed Corp., San Diego, CA) at home for one full night before the laboratory study. We excluded participants who had either an Apnea-Hypopnea Index (AHI) of 15 or greater (i.e. 15 or more sleep apnea-hypopnea events per hour of sleep), or an AHI of 5 or greater and at least one of the following: an ESS score of 10

or greater; awakenings with breath holding, gasping, or choking; reports of habitual snoring or breathing interruptions by a bed partner, or a medical or mental health condition associated with sleep apnea (hypertension, cognitive dysfunction, mood disorder, atrial fibrillation, etc.).

The laboratory study lasted for 2 consecutive nights and days. Participants arrived at 20:00 on Night 1 and were fitted with a 64-channel HydroCel Geodesic Sensor Net (Electrical Geodesics Inc., Eugene, OR) for EEG recording. We provided the participants with an 8-h sleep opportunity (23:00–07:00) and recorded EEG data throughout the entire night of sleep. On the morning of the next day (Day 1), we removed the sensor net from the participants and instructed them to perform multiple sessions of alertness and working-memory tests. We refitted the participants with the sensor net at 21:00 and repeated the same procedures on Night 2 and Day 2. We discharged the participants at 20:00 on Day 2.

Of the 85 participants that completed the study, 37 (31 men and 6 women) were diagnosed with PTSD and 48 (47 men and 1 women) without PTSD. Because sleep spindles differ between men and women [16, 28], and because there were six women in the PTSD group but only one woman in the non-PTSD group, we restricted our analyses to the 78 men. To evaluate the reproducibility of our findings, we split this sample into a discovery set comprising the first 47 consecutive participants (~60% of the total, 18 with PTSD) to obtain our initial findings, and a replication set comprising the remaining 31 participants (~40% of the total, 13 with PTSD) to assess the reproducibility of the initial findings.

Sleep EEG recordings and preprocessing

During the two study nights, we recorded 64-channel EEG data at a sampling rate of 250 Hz using the linked mastoids as the reference. We visually scored sleep stages and arousals in 30-s epochs for each night according to the criteria of the American Academy of Sleep Medicine [29]. We performed off-line data processing using custom scripts written in MATLAB (The MathWorks Inc., Natick, MA). After band-pass filtering the continuous EEG data at 0.5–50 Hz to eliminate noise and segmenting the data into 5-s epochs, we removed all epochs contaminated by muscle or ocular artifacts using previously validated algorithms [30–32].

Spindle detection and analysis

We used a previously established algorithm [33, 34] to separately detect slow (10–13 Hz) and fast spindles (13–16 Hz) at each EEG channel during NREM sleep stages N2 and N3 (Figure 1A). Briefly, we band-pass filtered the continuous EEG data in the slow- and fast-spindle frequency ranges separately and calculated the root-mean-square (RMS) of the filtered signal using a 250-ms moving window with a 25-ms moving step. We then applied a constant threshold based on the 95th percentile of the RMS amplitude from artifact-free N2 and N3 epochs of the entire night. We defined a spindle as an event where the RMS amplitude from artifact-free N2 and N3 epochs exceeded the threshold for a duration between 0.5 and 3.0 s, where we marked its beginning and end at the threshold-crossing points. Using the procedure above, we observed the expected topographical distributions for slow- and fast-spindle density (i.e. frontal

dominance for slow spindles and centro-parietal dominance for fast spindles; Figure 1B), and the density values were within the range of other studies [20, 35].

We investigated four spindle parameters: amplitude, duration, oscillatory frequency, and density. We computed these four parameters at each channel for slow and fast spindles. Not every participant showed spindles at channels near the edges of the 64-channel montage. This was expected because spindles are most prevalent over the central areas. Figures S1 and S2 show the numbers of participants used at each channel for the discovery and replication analyses, respectively.

Age correction

Previous studies have shown that sleep-spindle parameters change with age [16, 36]. Given that our PTSD participants were on average 3.6 years younger than the non-PTSD participants in the discovery analysis (Table 1), we performed a regression-based age correction to address the concern that this difference might confound our findings. Briefly, we used univariate regression analyses to examine associations between age and each spindle parameter at each channel. If the association between a given parameter and age was significant ($p < 0.05$) at any channel, we performed the correction for all channels by subtracting the product of age (zero-meaned) and its regression coefficient from the raw value of the parameter. We used only non-PTSD participants to determine the regression coefficients, to avoid the possibility that the use of PTSD participants might remove disease-related effects [37]. We used the method proposed by Green [38] to compute the regression coefficients, and performed age correction prior to statistical analyses.

Statistical analyses

We used the Wilcoxon rank-sum test to initially assess group differences in spindle parameters on a channel-by-channel basis. To account for multiple comparisons across channels, we performed a permutation-based test [7, 39]. Briefly, we created 10,000 permuted datasets by randomly shuffling the label of each participant in the two groups. For each permutation, we identified the largest cluster of neighboring channels where $p < 0.05$ for each channel in the cluster. We then used the number of channels in the largest cluster of each permutation to construct a "null" distribution and used this distribution to test whether the size of each cluster from the actual (correctly labeled) data was statistically significant. We performed this analysis for each of the computed spindle parameters. To further account for multiple comparisons across the eight parameters investigated (4 parameters × 2 spindle types), we performed Bonferroni corrections. To assess group differences in spindle parameters for different sleep cycles, we performed a two-way repeated-measures analysis of variance (rANOVA) with Group (PTSD and non-PTSD) as the between-subject factor and Sleep Cycle (cycles 1, 2, and 3) as the within-subject factor. We considered $p < 0.05$ as statistically significant.

Evaluation of reproducibility

One of our aims was to assess the reproducibility of our findings. As no single test can sufficiently describe whether a replication is
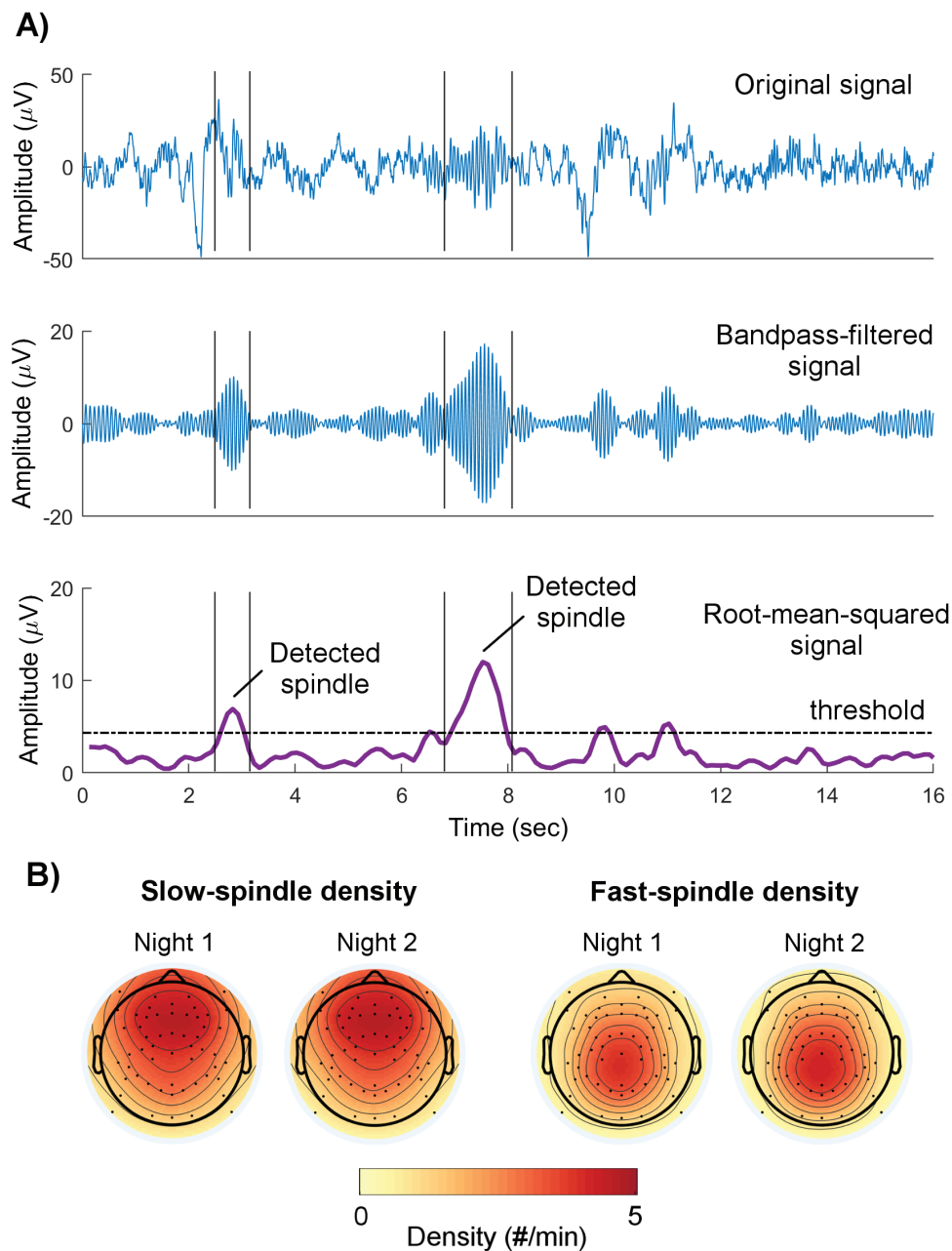
A)

B)

Figure 1. Illustration of the spindle-detection algorithm. Vertical black lines enclose detected spindles.

a success, we used three tests to evaluate reproducibility [40]. The first test assessed whether the replication analysis showed a statistically significant difference between the two groups ($p < 0.05$) in the same direction as the original finding. This is a commonly used test, which depends on the sample size and treats the $p < 0.05$ threshold as a red-line criterion between success and failure. The second test, which complemented the first, assessed whether the effect size of the replication analysis fell within the 95% confidence interval (CI) of the original finding. The third test, which assessed the effect sizes and statistical significance of the group differences for the discovery and replication data combined, provided information about the cumulative evidence of the discriminatory power of the parameter. We computed the effect size using a robust version of Cohen's *d*, constructed by replacing the population mean with a 20% trimmed mean and

the population standard deviation with the square root of a 20% winsorized variance [41]. We used a bootstrap approach with 10,000 replicates to determine the 95% CI of the effect sizes [42].

Results

Clinical characteristics and sleep architecture parameters of the discovery and replication sets

Table 1 shows the clinical characteristics of the discovery and replication sets. As expected, the CAPS, PHQ-9, ISI, and PSQI scores were higher in the PTSD group than in the non-PTSD group in both data sets (all values of $p < 0.001$). The ESS score was higher in the PTSD group than in the non-PTSD group in the discovery set ($p = 0.003$) but not in the replication set ($p = 0.856$).

Table 1. Clinical characteristics of the discovery and replication sets

Variable	Discovery set			Replication set		
	PTSD [$n = 18$] Mean (SD)	Non-PTSD [$n = 29$] Mean (SD)	Group Comparison P-value†	PTSD [$n = 13$] Mean (SD)	Non-PTSD [$n = 18$] Mean (SD)	Group Comparison P-value
Age (years)	29.9 (4.1)	33.5 (7.3)	0.118	33.1 (5.1)	31.7 (3.9)	0.535
CAPS	52.6 (15.9)	10.6 (7.8)	**<0.001**	49.8 (18.4)	5.4 (7.3)	**<0.001**
Intrusion	11.9 (4.7)	0.4 (1.3)	**<0.001**	9.0 (7.0)	0.8 (2.5)	**<0.001**
Avoidance	18.4 (8.6)	2.4 (4.1)	**<0.001**	14.8 (8.8)	0.5 (1.5)	**<0.001**
Hyperarousal	18.7 (7.9)	4.7 (4.3)	**<0.001**	19.5 (6.0)	1.1 (2.4)	**<0.001**
Nightmare item	2.1 (2.2)	0.2 (0.7)	**<0.001**	2.6 (2.2)	0.0 (0.0)	**<0.001**
PHQ-9	8.7 (5.0)	1.6 (2.6)	**<0.001**	5.8 (2.6)	1.4 (2.5)	**<0.001**
ISI	12.7 (4.6)	3.9 (4.1)	**<0.001**	14.2 (4.8)	3.8 (4.2)	**<0.001**
PSQI	9.3 (2.7)	4.1 (2.7)	**<0.001**	8.9 (2.8)	4.1 (2.4)	**<0.001**
ESS	8.3 (4.7)	4.5 (2.8)	**0.003**	5.8 (3.9)	5.7 (3.3)	0.856
AHI	1.9 (1.7)	2.7 (2.7)	0.409	4.1 (3.8)	2.0 (2.6)	0.054
AUD history‡ (n)	11	8	—	8	2	—
SUD§ history (n)	3	1	—	2	1	—

AHI, Apnea–Hypopnea Index; AUD, alcohol use disorder; CAPS, Clinician Administered PTSD Scale; ESS, Epworth Sleepiness Scale; ISI, Insomnia Severity Index; PHQ-9, Patient Health Questionnaire-9; PSQI, Pittsburgh Sleep Quality Index; SUD, substance use disorder.
†Wilcoxon rank-sum test.
‡Absent within at least the past 3 months.
§Assessed for sedatives, hypnotics, anxiolytics, cannabis, stimulants, opioids, cocaine, hallucinogens, phencyclidine, and poly-drugs. Bold values represent $p < 0.05$.

The AHI score did not differ between groups in both data sets ($p > 0.05$).

Regarding sleep architecture parameters, the only parameter that exhibited significant group differences ($p < 0.05$) in both the discovery and replication sets was the number of awakenings per sleep hour on Night 2 (Table 2). Several other sleep architecture parameters, including sleep latency, sleep efficiency, wakefulness after sleep onset, N2 sleep percentage, and N3 sleep percentage, exhibited significant group differences in the discovery set but not in the replication set.

Topographical analysis of sleep-spindle parameters (discovery analysis)

Figure 2 shows the topographical differences in spindle parameters between the PTSD ($n = 18$) and non-PTSD ($n = 29$) groups in the discovery set. The black dots indicate channels with $p < 0.05$ uncorrected for multiple comparisons; the white dots indicate channels that belong to a statistically significant cluster after accounting for multiple comparisons across channels.

Slow-spindle parameters.
Compared to the non-PTSD group, the PTSD group exhibited higher oscillatory frequency of slow spindles over the antero-frontal regions (Figure 2A, column 3). There were only two clusters, one with 10 channels on Night 1 and another with 17 channels on Night 2, which passed the initial statistical threshold (uncorrected $p < 0.05$). The permutation test which accounts for multiple comparisons across channels suggested that the Night 1 cluster of channels approached significance ($p = 0.084$, mean effect size = 0.89) and the Night 2 cluster of channels was statistically significant ($p = 0.046$, mean effect size = 0.92). The p-values were not below the Bonferroni-corrected threshold across the eight tested spindle parameters ($p = 0.05/8 = 0.006$). Slow-spindle amplitude, duration, and density did not differ between groups on either night (Figure 2A, columns 1, 2, and 4, respectively).

Fast-spindle parameters.
The oscillatory frequency of fast spindles was higher in PTSD participants than in non-PTSD participants over a broad centro-parietal area. This effect was not significant on Night 1 (Figure 2B, column 3, top), but was on Night 2 (on a single cluster of 35 channels, $p = 0.004$, mean effect size = 0.90; Figure 2B, column 3, bottom), with the p-value remaining significant after Bonferroni correction. Fast-spindle amplitude, duration, and density did not differ between groups on either night (Figure 2B, columns 1, 2, and 4, respectively).

Replication analysis

The main findings of the discovery analysis were that, compared to non-PTSD participants, PTSD participants showed (1) higher slow-spindle oscillatory frequency over the antero-frontal regions on both nights and (2) higher fast-spindle oscillatory frequency over the centro-parietal regions on Night 2. The aim of the replication analysis was to assess whether we could reproduce these findings in the reserved subsample of participants (replication set: 13 PTSD and 18 non-PTSD), judging by a set of three tests (see Methods). To this end, based on the topographical results of the discovery analysis (Figure 2), we selected an antero-frontal region of interest (ROI) and a centro-parietal ROI to assess slow- and fast-spindle frequencies, respectively. Figure 3 illustrates the ROIs and ROI-based group differences for the discovery, replication, and combined sets. Figure 4 shows the corresponding effect sizes.

The group difference in slow-spindle oscillatory frequency was in the same direction for the replication analysis as it was for the discovery analysis, and significant on Night 1 ($p = 0.014$) but not on Night 2 ($p = 0.155$) (Figure 3A). The effect sizes (Night 1, 1.07; Night 2, 0.72) fell within the 95% CI of the discovery effect sizes for both nights (Figure 4A). For the combined analysis, the group differences were significant on both nights (Night 1: $p = 0.002$, effect size = 0.72; Night 2: $p = 0.006$, effect size = 0.68).

Table 2. Sleep architecture parameters of the discovery and replication sets

Variable	Discovery set			Replication set		
	PTSD [n = 18] Mean (SD)	Non-PTSD [n = 29] Mean (SD)	Group Comparison P-value[†]	PTSD [n = 13] Mean (SD)	Non-PTSD [n = 18] Mean (SD)	Group Comparison P-value
Sleep latency (min)						
Night 1	16.6 (18.8)	13.8 (15.2)	0.346	19.2 (32.2)	7.2 (5.2)	0.138
Night 2	12.5 (11.1)	7.4 (6.6)	**0.042**	8.8 (6.7)	12.8 (21.1)	0.326
Sleep efficiency (%)						
Night 1	85.1 (7.5)	84.5 (8.1)	0.735	84.0 (7.5)	87.6 (5.2)	0.186
Night 2	84.6 (6.6)	89.5 (7.3)	**<0.001**	89.6 (3.9)	88.9 (7.6)	0.873
WASO (min)						
Night 1	54.2 (28.8)	60.4 (35.3)	0.751	57.5 (26.2)	52.2 (23.9)	0.631
Night 2	60.9 (31.5)	42.9 (34.2)	**0.002**	41.1 (22.9)	37.1 (27.9)	0.548
Number of awakenings per sleep hour						
Night 1	5.3 (2.0)	5.5 (2.3)	0.638	5.2 (1.7)	4.7 (1.9)	0.367
Night 2	5.5 (1.9)	4.7 (1.8)	**0.022**	5.3 (1.8)	3.9 (1.2)	**0.039**
REM density (counts/min)						
Night 1	5.3 (2.8)	5.4 (3.5)	0.991	2.0 (2.3)	2.5 (2.8)	0.659
Night 2	5.9 (3.5)	5.9 (4.0)	0.852	2.9 (3.4)	3.1 (3.6)	0.983
N1%						
Night 1	12.0 (5.4)	12.1 (6.5)	0.424	10.5 (3.7)	9.0 (3.7)	0.238
Night 2	10.1 (4.1)	9.5 (5.4)	0.090	9.1 (3.3)	7.2 (3.0)	0.105
N2%						
Night 1	58.0 (7.2)	55.9 (6.9)	0.090	57.1 (7.6)	55.4 (6.6)	0.617
Night 2	56.3 (6.4)	53.5 (6.6)	**0.026**	53.8 (8.1)	53.1 (6.4)	0.795
N3%						
Night 1	8.6 (6.3)	13.2 (7.4)	**0.004**	9.1 (7.5)	11.7 (5.3)	0.347
Night 2	10.6 (6.0)	14.4 (7.7)	**0.021**	11.3 (5.7)	12.8 (6.3)	0.920
NREM%						
Night 1	78.4 (6.3)	81.1 (5.6)	0.208	76.7 (4.7)	76.2 (4.1)	0.826
Night 2	77.0 (5.1)	77.4 (5.9)	0.686	74.2 (6.3)	73.0 (6.0)	0.617
REM%						
Night 1	21.6 (6.3)	18.9 (5.6)	0.208	23.3 (4.7)	23.9 (4.1)	0.826
Night 2	23.0 (5.1)	22.6 (5.9)	0.686	25.8 (6.3)	27.0 (6.0)	0.617

Bold values indicate $p < 0.05$.
WASO, wakefulness after sleep onset; NREM, nonrapid eye movement; REM, rapid eye movement.
[†]Wilcoxon rank-sum test after age correction.

These results satisfied all three tests for Night 1 and two of the three for Night 2, showing good reproducibility of our original findings on slow-spindle oscillatory frequency.

The group difference in fast-spindle oscillatory frequency on Night 2 for the replication analysis was not significant ($p = 0.435$) but in the same direction as it was for the discovery analysis (Figure 3B). Although the effect was small (effect size = 0.26), it fell within the 95% CI of the discovery effect size (Figure 4B). The group differences remained statistically significant for the combined analysis ($p = 0.020$, effect size = 0.54). These results satisfied two of the three tests, indicating a reproducible trend of our original finding on fast-spindle oscillatory frequency on Night 2. The group differences in fast-spindle oscillatory frequency on Night 1 were not statistically significant for both discovery and replication sets, but the trends were consistent and in the same direction as on Night 2 (Figure 3B).

Table S1 provides the ROI-based spindle oscillatory frequency values for the discovery and replication sets. To complement the ROI-based assessment of reproducibility, we show the topographical results from the discovery and replication analyses side-by-side, which allows for a visual assessment of reproducibility (Figure 5).

We conducted several additional analyses to verify the robustness of our findings. First, the results reported above were based on the combined analyses of N2 and N3 sleep spindles. We also examined N2 sleep spindles alone and found similar results (Figure S3). Second, to address the possibility that our findings may have been due to between-group differences in the number of EEG arousals (i.e. abrupt shifts in EEG frequency), we verified that the number of EEG arousals did not differ between the PTSD and non-PTSD groups during either NREM or REM sleep stages (all values of $p > 0.43$), and that our overall findings remained unchanged after removing arousals from the analysis (Figure S4). Third, to verify that our findings were robust to differences in the spindle-detection algorithm, we re-examined our data using a different spindle detector, described by Ferrarelli et al. [43]. Although this new algorithm detected fewer spindles than did our algorithm, the overall findings remained unchanged (Figure S5; see Table S2 for the number and characteristics of spindles detected by each algorithm). Furthermore, we verified that using different spindle-detection thresholds (i.e. the 92nd and 98th percentiles of the RMS amplitude; Figure S6) did not alter our overall findings obtained with a 95th percentile threshold. Finally, investigation of the four spindle parameters for the combined range of spindle frequencies (10–16 Hz), rather

PTSD vs. non-PTSD — discovery analysis

A) Slow-spindle parameters

B) Fast-spindle parameters
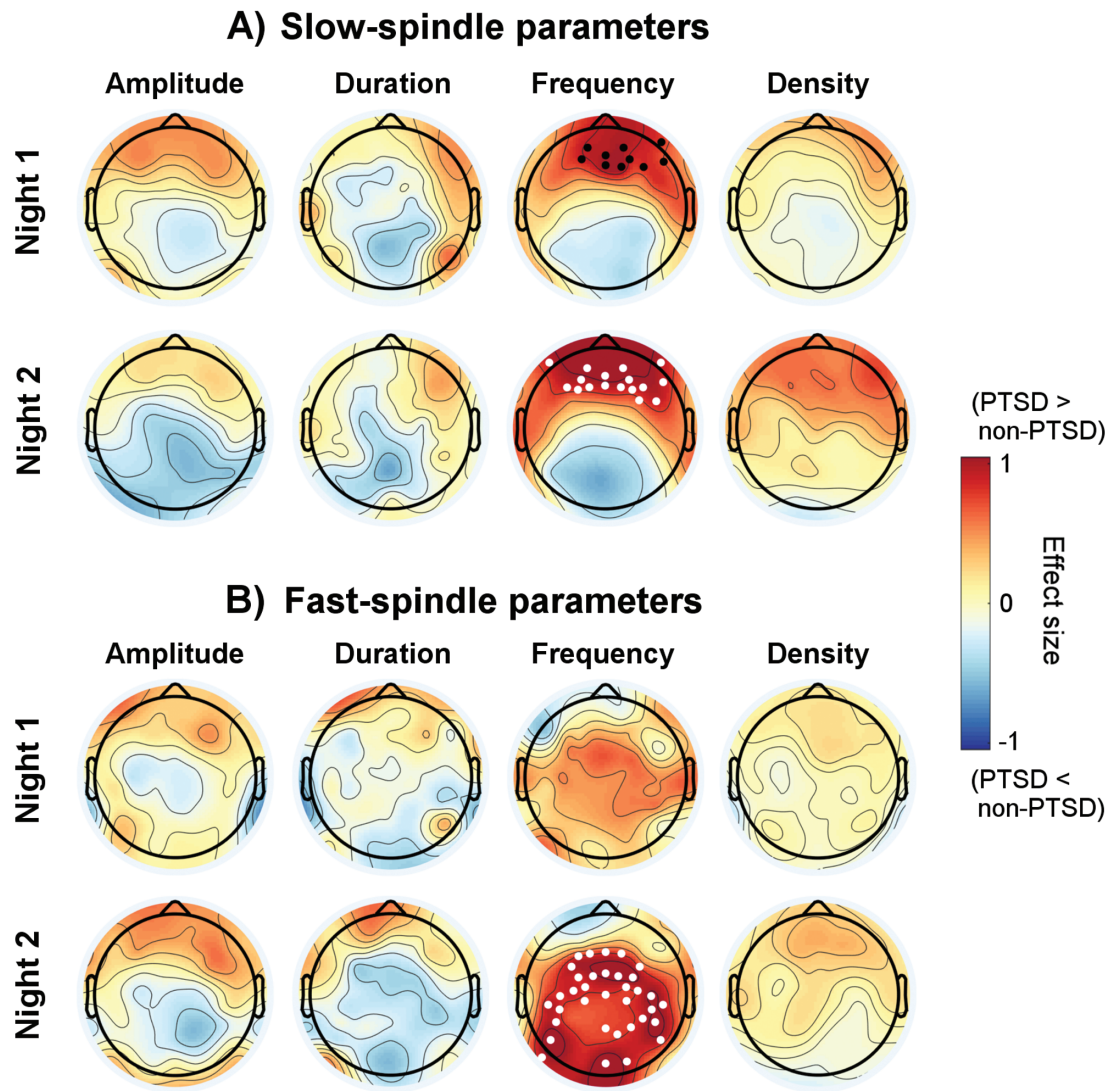
Figure 2. Topographical differences between the PTSD (n = 18) and non-PTSD (n = 29) groups in (A) slow- and (B) fast-spindle parameters for the discovery analysis. The heat maps show the individual-electrode effect size (a robust version of Cohen's d) for comparisons between PTSD and non-PTSD subjects. Blue areas show a decrease in spindle parameter in PTSD subjects relative to non-PTSD subjects (PTSD < non-PTSD), whereas red areas show an increase (PTSD > non-PTSD). Black dots indicate electrodes with uncorrected p-values less than 0.05. White dots indicate electrodes that belong to a statistically significant cluster (p < 0.05) after accounting for multiple comparisons across electrodes.

than for slow and fast spindles separately, also revealed similar findings (i.e. the oscillatory frequency of spindles was higher in the PTSD group than in the non-PTSD group; Figure S7).

Correlations between spindle oscillatory frequency and PTSD symptom severity

We explored the correlations (by Spearman's rho) between spindle frequencies (slow and fast, from the ROIs in Figure 3) and CAPS scores (total score, subscores for each symptom cluster, and nightmare item score) using the combined set (31 PTSD and 47 non-PTSD). The strongest correlation across all participants was between slow-spindle oscillatory frequency and the CAPS intrusion score (rho = 0.37, p < 0.001 for both nights). Slow-spindle oscillatory frequency was also significantly correlated with the CAPS total score (Night 1: rho = 0.27, p = 0.017; Night 2: rho = 0.27, p = 0.019), the CAPS avoidance score (Night 1: rho = 0.27, p = 0.017;

Night 2: rho = 0.28, p = 0.013), and the CAPS nightmare item score (Night 1: rho = 0.24, p = 0.034; Night 2: rho = 0.24, p = 0.036). Fast-spindle oscillatory frequency was significantly correlated with the CAPS intrusion score (rho = 0.22, p = 0.049) and the CAPS hyperarousal score (rho = 0.25, p = 0.029) only for Night 2. No other correlation was significant either across all participants or within a group. We note that only the correlation between slow-spindle oscillatory frequency and CAPS intrusion score remained significant after correcting for multiple comparisons using the Bonferroni method (corrected p < 0.01 for both nights)

Correlations between spindle oscillatory frequency and sleep maintenance

We further examined whether spindle frequencies were related to the increased number of awakenings per sleep hour (poor sleep maintenance) in the PTSD group using the combined set.
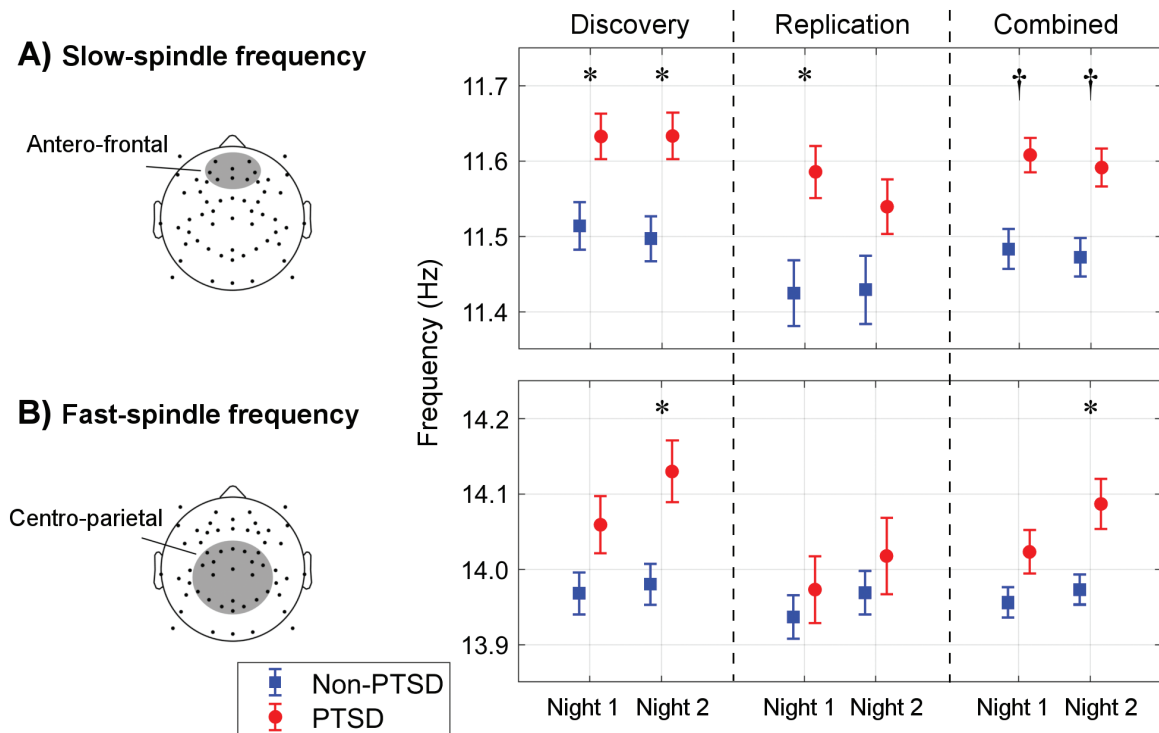
Figure 3. Group differences in (A) slow- and (B) fast-spindle frequencies for the selected regions of interest (ROIs) for the discovery (18 PTSD and 29 non-PTSD), replication (13 PTSD and 18 non-PTSD), and combined (31 PTSD and 47 non-PTSD) analyses. We selected the ROIs based on the topographical heat maps in Figure 2, with an antero-frontal ROI and a centro-parietal ROI selected to show differences in slow- and fast-spindle frequencies, respectively. We computed ROI-based measures by averaging electrode values within the ROIs. The plotted values are the group means of the ROI-based measures. Error bars indicate one standard error of the mean. Asterisks (*) indicate statistically significant group differences at $p < 0.05$, while daggers (†) indicate statistically significant group differences at $p < 0.05$ after correcting for multiple comparisons across the eight tested spindle parameters.
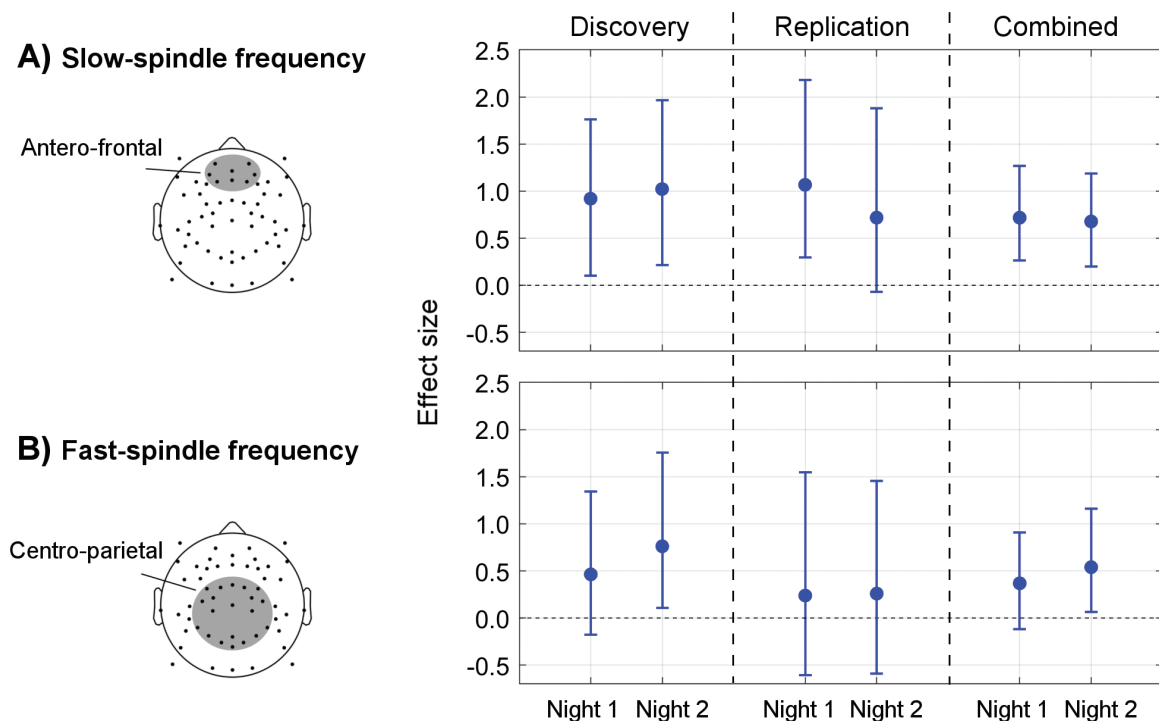
Figure 4. Effect sizes of the group differences in (A) slow- and (B) fast-spindle frequencies for the discovery, replication, and combined analyses. The plotted values indicate the effect size (a robust version of Cohen's *d*) for the selected regions of interest. Positive values indicate that spindle oscillatory frequency was higher in the PTSD group than in the non-PTSD group. Error bars indicate the 95% confidence intervals (CIs) of the effect sizes; the horizontal dashed lines indicate an effect size of zero. A 95% CI that does not cross zero implies the effect is significant at the 0.05 level.

We found that PTSD participants with higher fast-spindle oscillatory frequency tended to have more awakenings per sleep hour (Figure 6A, Night 1: Spearman's rho = 0.29, p = 0.111, Pearson's r = 0.48, p = 0.006; Night 2: Spearman's rho = 0.21, p = 0.250, Pearson's r = 0.385, p = 0.032). We observed no such trend for slow-spindle oscillatory frequency (Figure 6B).

Sleep-spindle oscillatory frequency across sleep cycles

We separately examined group differences in slow- and fast-spindle mean frequencies (from the two ROIs in Figure 3) for the first three sleep cycles using the combined set (Figure 7). For slow-spindle oscillatory frequency, a rANOVA revealed a significant main effect of Group for Night 1 ($F_{1,74}$ = 8.9, p = 0.004) and Night 2 ($F_{1,74}$ = 9.0, p = 0.004). For fast-spindle oscillatory frequency, we identified a significant main effect of Group for Night 2 ($F_{1,74}$ = 6.8, p = 0.011) but not for Night 1 ($F_{1,74}$ = 1.2, p = 0.269). None of the Group × Sleep Cycle interactions were significant, suggesting that the group differences in slow- and fast-spindle mean frequencies were similar across the first three sleep cycles. In contrast, we observed no significant group differences in slow- or fast-spindle density for the first three sleep cycles (Figures S8 and S9).

Discussion

This study aimed to investigate whether sleep spindles are modified in individuals with PTSD. We found that the oscillatory frequency of slow spindles was higher in PTSD participants than in non-PTSD participants, despite the absence of any significant group difference in spindle amplitude, duration, or density. Importantly, the finding was consistent across nights and its

trend was reproducible across subsamples of our study population. These results suggest that increased oscillatory frequency of slow spindles may be a neural correlate of PTSD during sleep.

Studies of sleep spindles in PTSD and other conditions

There is only one study to date that has directly examined sleep spindles in PTSD subjects [18]. Consistent with our observations, the study reported that PTSD and control subjects did not differ in spindle density. Unfortunately, the study did not examine other spindle parameters (i.e. amplitude, duration, and oscillatory frequency) beyond spindle density. A few other studies [7, 44, 45], including one from our own group [7] that used the same sample as in this study, have investigated PTSD-related changes in spindle activity indirectly by assessing sigma power during NREM or N2 sleep, which is highly correlated with spindle amplitude and density [16]. These studies have consistently reported that individuals with and without PTSD do not differ significantly in sigma power, in line with the present observations that spindle amplitude and density are unaltered in PTSD.

Although no prior study has investigated the oscillatory frequency of sleep spindles in PTSD, Picard-Deland et al. [46] examined spindle oscillatory frequency along with other spindle parameters in subjects who had frequent nightmares–a characteristic symptom of PTSD. They found that, compared to controls, these subjects showed reduced density of slow spindles in most brain regions and elevated oscillatory frequency of fast spindles in central brain regions. The pattern of their findings for the oscillatory frequency of fast spindles is similar to that observed in our study, raising the possibility that elevated fast-spindle frequency may be a neural correlate of the pathophysiology of nightmares shared by PTSD subjects and frequent nightmare sufferers. However, in our data, there was no significant correlation between
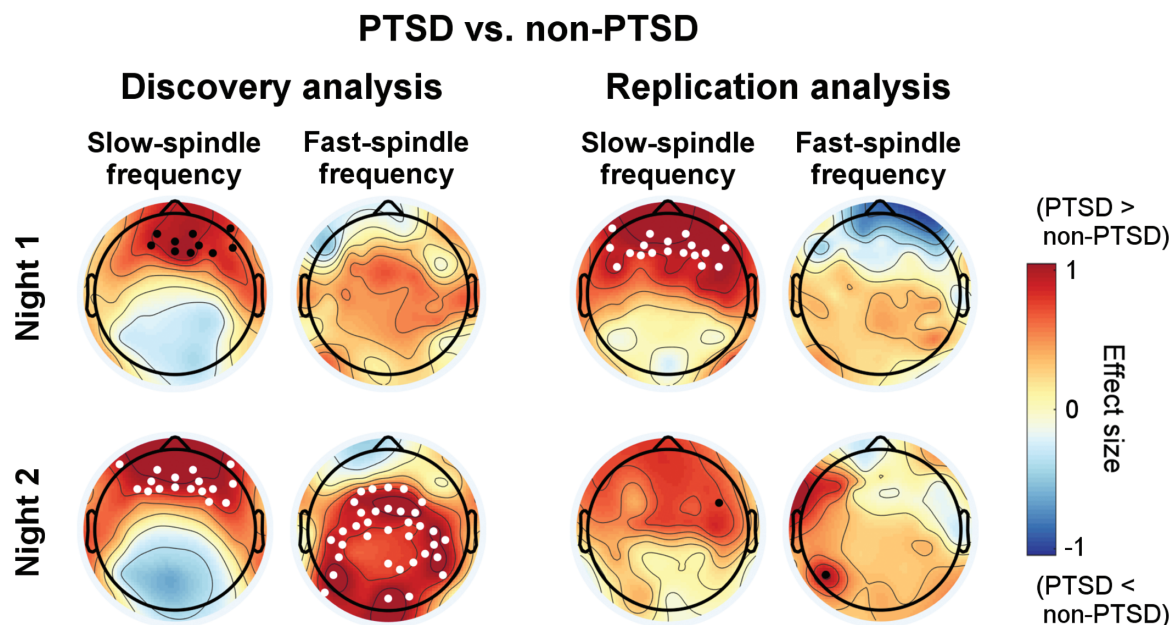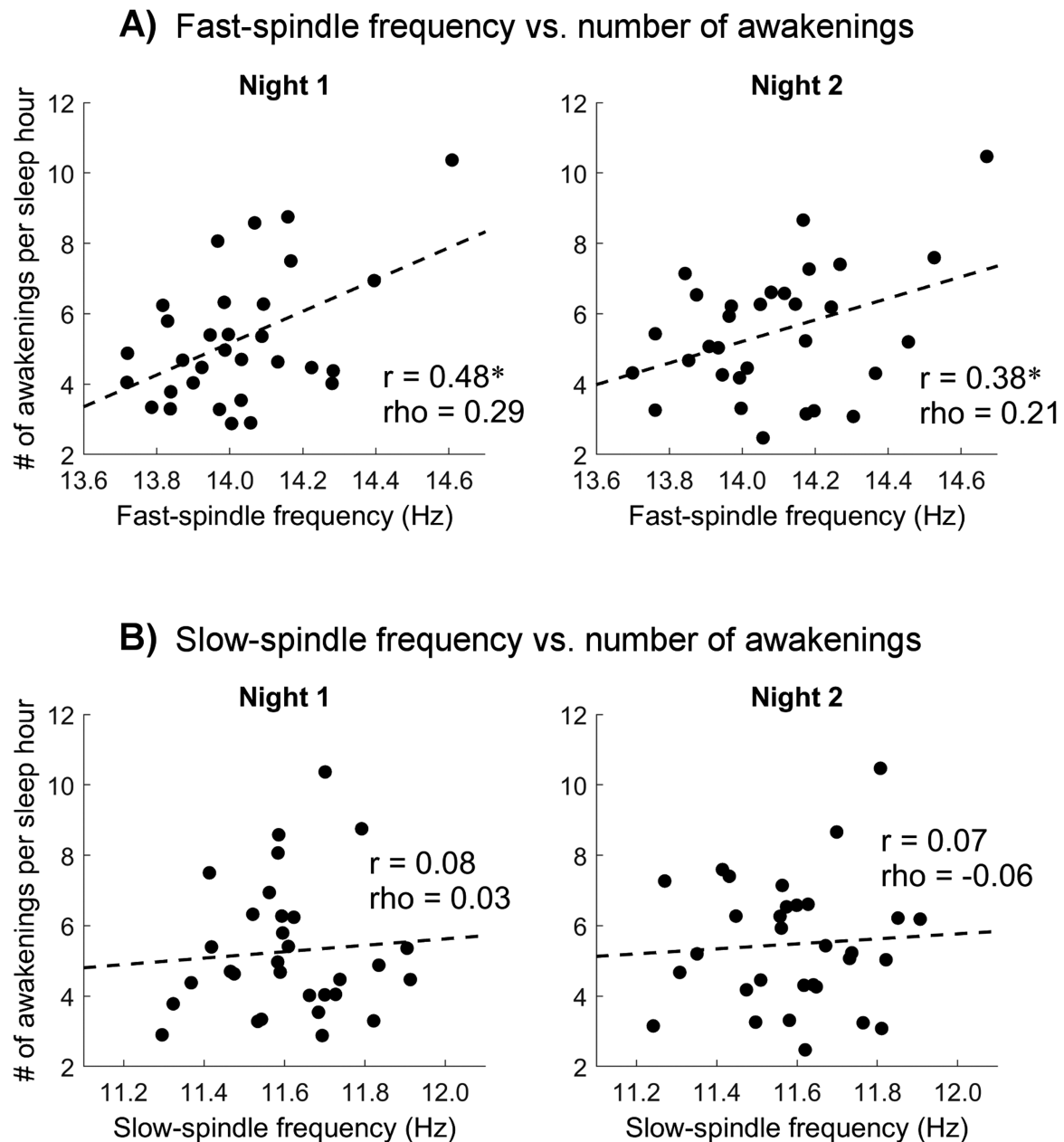

Figure 5. A side-by-side comparison of the results from the discovery analysis (18 PTSD and 29 non-PTSD) and the replication analysis (13 PTSD and 18 non-PTSD). The topographical heat maps show the individual electrode effect size (a robust version of Cohen's d) between the PTSD and non-PTSD groups. Black dots indicate electrodes with uncorrected p-values less than 0.05. White dots indicate electrodes that belong to a statistically significant cluster (p < 0.05) after accounting for multiple comparisons across electrodes.

A) Fast-spindle frequency vs. number of awakenings

B) Slow-spindle frequency vs. number of awakenings

Figure 6. Scatterplots showing correlations between (A) fast- and (B) slow-spindle oscillatory frequency (from the regions of interest in Figure 3) and the number of awakenings per hour of sleep among all PTSD participants (*n* = 31). Correlations are quantified by Pearson's *r* and Spearman's rho. Asterisks (*) indicate statistically significant correlation coefficients (*p* < 0.05).

the oscillatory frequency of fast spindles and the CAPS nightmare severity score, as would be expected if this were true. Moreover, although fast-spindle frequency differed between groups in the discovery analysis, it did not significantly differ in the replication analysis, underscoring the uncertainty of this finding.

Sleep-spindle alterations have been reported in several other disorders that are often comorbid with PTSD, including depression [47], schizophrenia [43, 48, 49], and insomnia [50]. However, the spindle changes we observed in PTSD subjects (who were free of these comorbidities) differ from those seen in these studies. Specifically, major depression is associated with increases in frontal and parietal spindle density [47]. In contrast, schizophrenia is often associated with reduced spindle density [49]. Lastly, patients with paradoxical insomnia exhibit no change in spindle density but a

decrease in spindle duration [50]. Although alterations in sleep-spindle oscillatory frequency have not been demonstrated in any of these disorders, few studies have explicitly examined the frequency parameter of sleep spindles, and a number of other comorbidities in PTSD, including AUD, generalized anxiety disorder, and bipolar disorder, have not been evaluated for changes in spindles. Therefore, until more evidence becomes available, the specificity of the present findings remains unclear.

Possible implications of increased spindle oscillatory frequency in PTSD

Although oscillatory frequency is one of the defining features of sleep spindles, its functional significance is not well established.

A) Slow-spindle frequency

B) Fast-spindle frequency
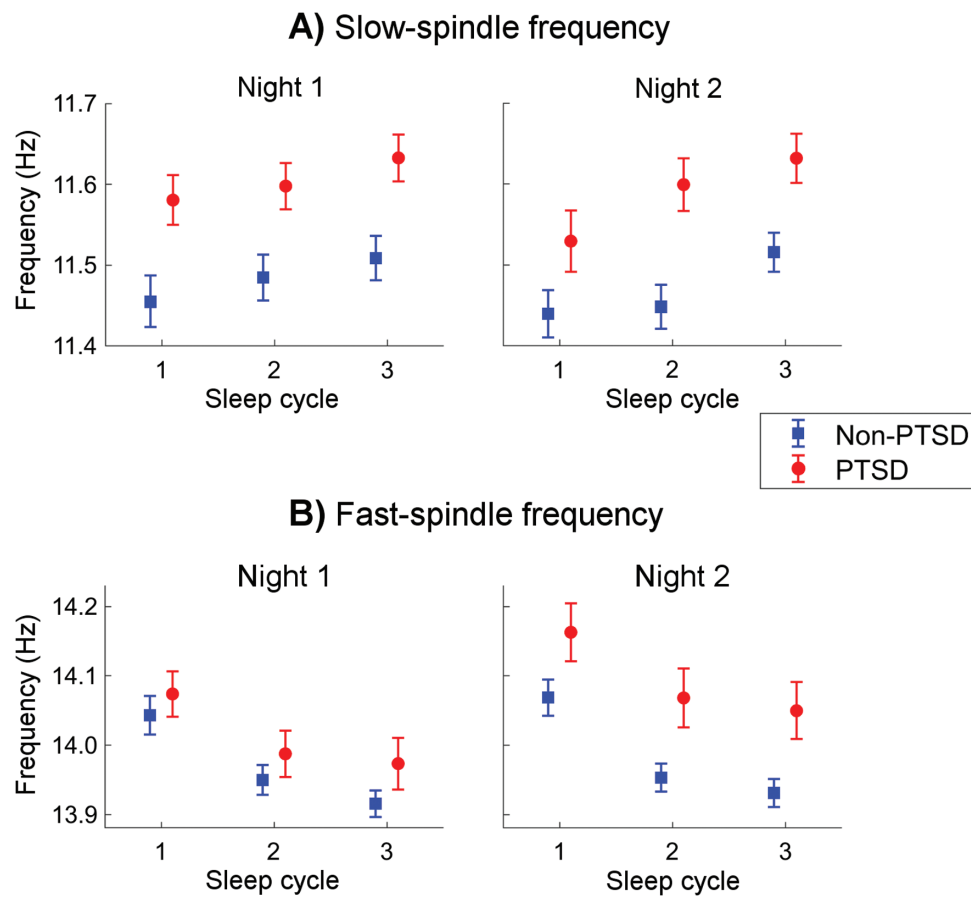
Figure 7. Group differences in (A) slow- and (B) fast-spindle frequencies for the first three sleep cycles using the combined data set (31 PTSD and 45 non-PTSD, 2 non-PTSD participants were excluded from this analysis due to lack of spindles in one of the sleep cycles). Error bars indicate standard errors of the mean.

Theoretically, the reciprocal interactions between reticular thalamic neurons and thalamocortical neurons are responsible for generating spindle waves [11, 51]. In such interactions, activated reticular thalamic neurons send inhibitory signals to large numbers of thalamocortical neurons through their divergent axonal projections, leading to hyperpolarization (inhibition) of thalamocortical neurons. Then, after a time delay, some of the inhibited thalamocortical neurons undergo post-inhibitory rebound firing, causing the re-activation of the reticular thalamic neurons and the initiation of the next cycle of spindle oscillation. As such, the oscillatory frequency of spindles is largely determined by the latency of post-inhibitory rebound firing of thalamocortical neurons, which is associated with the amplitude and duration of hyperpolarization [52]. Along these lines, Andrillon et al. [53] found that deep sleep (during which thalamic hyperpolarization is presumably strong) is associated with a reduction in spindle oscillatory frequency, leading them to suggest that spindle frequency is a state-like indicator reflective of the underlying level of thalamocortical hyperpolarization. According to this view, the increased spindle oscillatory frequency in PTSD participants observed here may indicate that the overall level of thalamocortical inhibition during NREM sleep was lower in PTSD subjects than in healthy controls. Given that one of the main functions of the thalamus during sleep is to stop relaying sensory information to the cortex and, thereby, protect the sleeping brain from disruptive stimuli [54], the reduced thalamocortical inhibition in PTSD could indicate a reduction in the ability of the thalamus to

block the flow of sensory information to the cortex and, hence, a deficiency in its ability to protect sleep. Indeed, we observed that, compared with non-PTSD participants, PTSD participants experienced more frequent awakenings after sleep onset during Night 2 for both discovery and replication sets (Table 2). Moreover, the oscillatory frequency of fast spindles tended to correlate positively with the number of awakenings per sleep hour among PTSD participants (Figure 6A). However, slow-spindle oscillatory frequency did not correlate with the number of awakenings (Figure 6B), even though the group differences in slow-spindle oscillatory frequency were more consistent across nights and subsamples.

Alternatively, sleep-spindle oscillatory frequency may be a trait-like indicator that reflects certain intrinsic properties of the thalamocortical network. Although spindle characteristics, such as density and oscillatory frequency, vary substantially between individuals, they are stable within individuals from night to night [16]. In our data, the intraclass correlation coefficients between the two study nights for slow- and fast-spindle frequencies were 0.92 and 0.90, respectively (Figure S10). This intra-individual stability suggests that sleep-spindle characteristics may reflect the anatomical or functional traits of an individual's underlying thalamocortical system [55]. Indeed, individual differences in white-matter microstructure, including axons surrounding and intrinsic to the thalamus, are correlated with spindle power and density [56]. However, it is currently unclear what network properties the slow- and

fast-spindle frequencies reflect. Furthermore, it remains to be determined whether elevated spindle oscillatory frequency is a risk factor of PTSD following trauma exposure, a consequence of PTSD pathology, or both.

Differences between the two nights of study

The group difference in fast-spindle oscillatory frequency was significant for Night 2 but not for Night 1 in the discovery analysis, although we observed the same trend in both nights (Figure 3). As we did not provide participants with an adaptation night before the two nights of laboratory study, the results of the first night may have been affected by differences in adaptation between the two groups. Additionally, participants had to perform multiple sessions of alertness and working-memory tests during the daytime preceding Night 2 but not during the daytime preceding Night 1. Given that fast-spindle activity is sensitive to previous learning experiences [57, 58], the difference in daytime activity may have contributed to the cross-night difference in the fast-spindle findings. Interestingly, in the replication analysis, although we observed similar trends, the group differences were not statistically significant on both nights, perhaps because of the small sample size. In contrast to the findings in fast-spindle oscillatory frequency, the findings in slow-spindle oscillatory frequency were more stable across nights, suggesting that this parameter is less sensitive to adaptation effects and previous daytime experiences.

Limitations

First, our study sample consisted of only men. The extent to which the present findings are generalizable to women needs to be evaluated in future studies. It is noteworthy that in women sleep-spindle parameters can change during the menstrual cycle [59]. Additionally, the prevalence of a history of AUD was higher among PTSD participants (~60%) than among non-PTSD participants (~20%). Alcoholism can affect sleep even after cessation of drinking [60]. It remains unclear whether a history of AUD affects sleep spindles. Nevertheless, when controlling for AUD history, our findings remained significant. Finally, the present study focused on sleep spindles without considering their temporal dynamics in relation to other sleep-related EEG patterns, such as slow oscillations and delta waves. Previous studies have shown that the temporal coupling between spindles and slow oscillations is essential for memory consolidation [61, 62]. The recent work by Kim et al. [63] further suggests that slow oscillations and delta waves may have competing roles, with the former enhancing memory consolidation and the latter promoting the opposite. It is yet to be determined whether interactions among sleep spindles, slow oscillations, and delta waves are altered in PTSD.

Conclusions

In summary, we have demonstrated that the oscillatory frequency of slow spindles is higher in participants with PTSD when compared with controls. The finding has implications for understanding the pathophysiology underlying the reported sleep disturbances in PTSD. In addition to independent validation, future studies with a longitudinal design are needed to unveil whether sleep-spindle oscillatory frequency is a state-like marker appropriate for monitoring disease course and treatment outcome, or a trait-like marker that serves as a risk factor for identification of individuals prone to PTSD following trauma exposure.

Supplementary material

Supplementary material is available at *SLEEP* online.

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