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Authors

Campbell, Ian G

Zhang, Zoey Y

Grimm, Kevin J

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Sleep restriction effects on sleep spindles in adolescents and relation of these effects to subsequent daytime sleepiness and cognition.

Ian G Campbell[1], Zoey Y Zhang[1], Kevin J. Grimm[2]

[1]University of California Davis, Department of Psychiatry and Behavioral Sciences, Sacramento, CA 95817 USA

[2]Arizona State University, Department of Psychology, Tempe, AZ 85287 USA

Work performed at University of California Davis

Corresponding Author:

Ian G. Campbell, PhD

UC Davis Department of Psychiatry and Behavioral Sciences

2230 Stockton Blvd

Sacramento, CA 95817

igcampbell@ucdavis.edu

Dedication: This manuscript is dedicated to Irwin Feinberg. MD who passed away August 25, 2022.

Abstract

Study Objectives: Limiting spindle activity via sleep restriction could explain some of the negative cognitive effects of sleep loss in adolescents. The current study evaluates how sleep restriction affects sleep spindle number, incidence, amplitude, duration, and wave frequency and tests whether sleep restriction effects on spindles change across the years of adolescence. The study determines whether sleep restriction effects on daytime sleepiness, vigilance, and cognition are related to changes in sleep spindles.

Methods: In each year of this three year longitudinal study, 77 participants, ranging in age from 10 to 16 years, each completed three different time in bed (TIB) schedules: 7, 8.5 or 10 hours in bed for four consecutive nights. A computer algorithm detected and analyzed sleep spindles in night 4 central and frontal electroencephalogram (EEG). Objective and subjective daytime sleepiness and cognition were evaluated on the day following the fourth night.

Results: For 7h vs. 10h TIB average all-night frontal and central spindle counts were reduced by 35% and 32% respectively. Reducing TIB also significantly decreased spindle incidence in the first 5h of NREM sleep, produced small but significant reductions in spindle amplitude, and had little to no effect on spindle duration and spindle wave frequency. Sleep restriction effects did not change with age. The reductions in spindle count and incidence were related to daytime sleepiness on the following day but were not related to working memory.

Conclusion: The sleep loss effects on daytime functioning in adolescents is partially mediated by reduced sleep spindles impacting daytime sleepiness.

Key words: sleep loss, electroencephalogram, maturation, adolescence, sleep spindles, sleepiness

Statement of Significance

Due to their proposed role in memory and cognition, reduction in sleep spindle activity may contribute to the negative effects of sleep restriction in adolescents. Our within-subject dose-response study found that restricting time in bed reduced all night spindle count and the rate of spindle production. The spindle count reduction was related to increased sleepiness on the following day, and it significantly mediated the relation between nighttime sleep duration and daytime sleepiness. Sleep spindle count was unrelated to subsequent cognitive performance. Our study demonstrates that restricting time in bed is an effective approach for studying the relation between sleep spindles and other outcome measures, and we provide statistical methods for isolating sleep spindle effects from non-specific sleep duration effects.

Introduction

Brain maturation during adolescence involves reorganization of neural circuits via growth of white matter (myelination) and pruning of superfluous synapses. These brain maturation process can be measured anatomically [1-3], biochemically [4], and perhaps most clearly via electrical potentials [5-7]. Delta (1-4 Hz) EEG power during non-rapid eye movement (NREM) sleep decreases by more than 60% between ages 12 and 16 years [6]. The sleep EEG changes are not limited to slow wave activity. Power in the sigma (11-15 Hz) band of NREM sleep EEG also declines steeply between ages 12 and 16 years [8], and the frequency at which sigma power is maximum increases linearly across adolescence [7-9]. These changes in changes in sigma EEG are likely a result of changes in sleep spindles.

Sleep spindles are brief (0.25 to 1.5 s) clusters of 11-15 Hz waves, roughly sinusoidal in shape that occur during (NREM) sleep. They are crucial for distinguishing NREM sleep from the other two states of spontaneous brain activity, wake and REM sleep. A number of functions have been proposed including protection of the sleep state by blocking ascending sensory disturbances from reaching the cortex (reviewed in [10]) and memory consolidation during sleep (reviewed in [10,11]). The majority of evidence for a role of sleep spindles in memory consolidation is correlational. Spindles increase in sleep following learning, and the magnitude of the increase is correlated to subsequent performance of the task or to the retention of memory [11]. Although most studies have examined the association between spindles and memory processing after learning, spindles may also promote subsequent learning and cognitive ability [12,13].

Spectral analysis evaluation of sleep restriction effects on the sleep EEG of adolescents age 10 to 16 years found that sigma (11-15 Hz) was the frequency band most strongly affected [14]. Reducing time in bed from 10 to 7 hours decreased all night sigma energy by 40% and decreased sigma power in the first 5 hours of NREM by 12%. In other words, sleep restriction reduced both the all night accumulation of sigma activity and the rate of sigma production. The sigma power reduction likely reflects a decrease in sleep spindle activity. Reduced spindle activity may contribute to sleep restriction impairment of cognitive ability in adolescents. A recent meta-analysis found a positive relation between spindles and cognition in adolescents [15]. We recently documented the changes in sleep spindle measures across age 6 to 18 years [16]. Spindle wave amplitude decreases steeply between ages 12 and 16 years, incidence peaks at age 15 years, and frequency increases linearly across childhood and adolescence. We interpreted these changes in spindle measures as maturation of thalamocortical circuits and a decrease in sleep depth.

In sleep following 40 h of sleep deprivation, sleep spindle incidence decreased and spindle wave amplitude increased in young adults [17]. A recent between-subjects study of adolescents found that sleep restriction decreased spindle amplitude and increased spindle duration [18]. Here we analyze our early adolescent dataset to evaluate within-subjects how sleep reduction affects sleep spindle measures. We also determine whether sleep restriction effects on sleep spindle measures change across the 10 to 16 year age range of this study. We examine whether sleep restriction induced reduction in the number of spindles is associated with changes in daytime sleepiness and performance. We test whether objective and subjective daytime sleepiness, sustained

vigilance, working memory scanning efficiency, and resistance to proactive interference are related to frontal and/or central spindles in the prior night's sleep. Finally, we assess whether spindles mediate the effect of prior sleep duration on these sleepiness and performance measures.

Methods

Experiment Design

A total of 77 subjects enrolled in this three-year longitudinal study, including 41 male and 36 female children ranging in age from 9.9 to 16.2 years (mean = 13.2). Annually each subject completed the following time in bed (TIB) conditions: 7 hours, 8.5 hours, and 10 hours for four consecutive nights. TIB was altered by moving the bedtime; subjects kept their habitual weekday rise time for all three TIB conditions. Three nights with 8.5 hours in bed preceded each 4-night TIB condition. All night EEG was recorded in the subjects' homes on the 2nd and 4th night of the 4-night TIB schedule. EEG was recorded (400 Hz digitization rate) on Grass Aura24 recorders using following electrodes: F3, F4, C3, C4, P3, P4, O1, O2, A1, A2, LOC, ROC, forehead, two chin locations and reference and ground electrodes on the scalp and forehead. We present results for only night-4 when the effects of the TIB schedule would be greatest.

On the weekend morning following the 4th night of the assigned TIB schedule, subjects reported to the sleep lab for a day of performance and sleepiness testing. Subjects completed four testing blocks (9 AM, 11 AM, 1 PM, 3 PM) with about an hour break between these blocks. Within each testing block, subjects completed a 9 minute waking EEG recording, subjective sleepiness ratings on the Karolinska Sleepiness Scale (KSS), a psychomotor vigilance task (PVT), and a multiple sleep latency test (MSLT). In

addition, in the 11 AM and 3 PM testing blocks, the subjects completed a modified Sternberg test [19]. The KSS is a 9 point scale ranging from 1 – “very alert” to 9 – “very sleepy (fighting sleep)” [20]. In each testing block, subjects completed a KSS prior to the waking EEG recording and another prior to the MSLT. The PVT, performed on a laptop computer, was 10 minutes in duration with inter-trial intervals ranging from 2 to 10 seconds. The PVT outcome measure was the log of the signal to noise ratio [21]. The more common measure, number of lapses greater than 500 ms, is not appropriate for this age group because the mean response time was greater than 500 ms for some of the youngest subjects [22]. For the MSLT, subjects lay down in bed and at lights off were asked to make themselves comfortable and to try to fall asleep [23]. The EEG was monitored during the MSLT, and subjects were woken after 5 consecutive 20 second epochs of stage N1 or a single epoch of stage N2, N3, or REM sleep. The test was concluded upon waking the subject or after 20 minutes if the subject did not fall asleep. During the modified Sternberg test, subjects were shown a set of either 2 or 4 letters to hold in memory. They were then shown a probe letter and instructed to respond as quickly and accurately as possible whether or not the probe letter was part of the memory set. The slope of the function describing the relation between response time and memory set size is a measure of working memory scanning efficiency [24]. Half of the probes that were not in the memory set were in the previous memory set. The slower response time for these recent probes is a measure of the effect of proactive interference [19].

More details of this study, including subject recruitment, subject retention, study design, and Aura hardware filters, can be found in our previous publications [22,25]. The data

underlying this article will be shared in response to reasonable requests to the corresponding author. The UC Davis Institutional Review Board approved all study procedures.

Spindle Detection and Analysis

Prior to spindle analysis, each 20 second epoch of nighttime EEG recordings was scored for sleep stage using modified [25] 2007 AASM standards [26]. For epochs scored as N2 or N3, spindles were detected and analyzed with a MATLAB program adapted from the program used by Goldstone et al [27] which is based on spindle detection techniques developed by Bodizs et al [28]. Details of the program can be found in Zhang et al [16]. Prior to analysis the frontal channel (F3-A2 or F4-A1) and central channel (C3-A2 or C4-A1) with fewer artifacts was selected. The program first identified the individual fast and slow spindle frequency range for each EEG recording by +/- 1.5 Hz of the sigma peak frequency of central and frontal channels. Then the raw EEG of both central and frontal was bandpass filtered by using the fast and slow spindle frequency range. Next, a Hilbert transformation was applied to the bandpass filtered EEG for generating the instantaneous amplitude envelope. A spindle detection threshold, which was defined as 3 standard deviations above the mean of Hilbert envelope, was used to detect sleep spindles for both central and frontal channels.

The following measures were calculated for each detected sleep spindle: the average peak trough amplitude, Hilbert amplitude, average frequency of waves within the spindle, and spindle duration. The detailed steps of calculations of these spindle measurements were in our previous publication [16]. The total number of spindles and the frequency at which sigma power was maximum was determined from all epochs of

NREM sleep in the entire night. For measures of amplitude, duration, and wave frequency, data were averaged over the first 5 hours of NREM sleep and the last 5 hours of NREM sleep, durations reached in all three TIB conditions. For the 5 hour NREM analyses we also determined spindle density (spindles per minute).

Statistical Analyses

We tested for effects of TIB, age, and age by TIB interaction on each spindle measure with mixed effects analysis [29] with TIB as a class variable and age as a continuous variable. Subject ages were centered by subtracting the average age, 13.2 years. Although our previous analyses of our large maturational dataset established non-linear age trends for the spindle measures [16], only linear age effects were evaluated in this smaller dataset. Logs of peak trough amplitude and Hilbert amplitude were used for statistical analyses because the raw amplitudes were not normally distributed. Rather than adding site as a factor variable, effects on frontal and central spindles were analyzed separately. We tested for the effects of night-4 sleep duration on spindle measures in separate analyses that replaced TIB with total sleep time (TST), treated as a continuous measure centered at its mean.

As described below, all night spindle count and spindle density in 5 h of NREM sleep were the spindle measures most strongly affected by the TIB schedule. We used multilevel models (SAS PROC MIXED and GLIMMIX) to evaluate the relation between these two measures and the following daytime sleepiness and performance measures: MSLT sleep likelihood, KSS ratings, log of the signal to noise ratio (LSNR) calculated for each 10-minute PVT, Sternberg slope, and Sternberg recency difference. Spindle count and density increased with sleep duration (TST). In an analysis of the spindle

count or density effect on sleepiness and performance measures, spindle count or density would simply be a proxy for sleep duration. Therefore, all analyses accounted for the effects of TST. Below we describe in detail the statistical analysis because it provides a method for isolating spindle effects from non-specific sleep duration effects. Multilevel mediation was evaluated through the estimation of two multilevel models. The multilevel models accounted for the different TIB conditions (t) within a year (i) and years nested within subjects (j). We review the specification using KSS as the primary outcome. The multilevel model for KSS is written as

Level 1 (TIB conditions within-year):

$$KSS_{tij} = \beta_{0ij} + \beta_1 \cdot SPN_{tij} + \beta_2 \cdot TST_{tij} + e_{tij}$$

Level 2 (between-years, within-person):

$$\beta_{0ij} = \gamma_{0j} + \gamma_1 \cdot SPN_{.ij} + \gamma_2 \cdot TST_{.ij} + d_{ij}$$

Level 3 (between-person):

$$\gamma_{0j} = \pi_0 + \pi_1 \cdot SPN_{..j} + \pi_2 \cdot TST_{..j} + s_j$$

where KSS_{tij} is participant j's KSS rating for condition t in year i. In the level-1 model, there is a random intercept (β_{0ij}) which represents participant j's conditional mean KSS rating in year i, the effect of spindles (β_1) and total sleep time (β_2) on night 4 of condition t in year i for individual j. The number of spindles and the total sleep time in the level-1 model represent individual deviations from the mean number of spindles and total sleep time for condition t in year i. In the level-2 model, the random intercept in year i for individual j is a function of an intercept for individual j (γ_0) representing the conditional mean KSS rating for individual j, the effect of spindles in year i for individual j (γ_1), and the effect of total sleep time in year i for individual j (γ_2). The number of

spindles and the total sleep time in the level-2 model are deviations from the mean for individual j. In the level-3 model, the random intercept for individual j is a function of a conditional grand mean (π_0) and the effects of the number of spindles (π_1) and total sleep time (π_2) for participant j. The number of spindles and the total sleep time in the level-3 model are the means of these variables for individual j.

A three-level model was then specified for the number of spindles given its potential mediating role between total sleep time and our outcomes. The three-level model for the number of spindles is written as

Level 1 (within-year):

$$SPN_{tij} = \delta_{0ij} + \delta_1 \cdot TST_{tij} + e_{tij}$$

Level 2 (between-years, within-person):

$$\delta_{0ij} = \zeta_{0j} + \zeta_1 \cdot TST_{.ij} + d_{ij}$$

Level 3 (between-person):

$$\zeta_{0j} = \theta_0 + \theta_1 \cdot TST_{..j} + s_j$$

where SPN_{tij} is the number of spindles on night 4 of recording t in year i for individual j, which is a function of the random intercept (δ_{0ij}) and total sleep time on night 4 of recording t in year i for individual j (δ_1) in the level-1 model. In the level-2 model, the random intercept is a function of the random intercept for individual j (ζ_{0j}) and total sleep time in year i for individual j (ζ_1). Finally, in the level-3 model, the random intercept is a function of the conditional grand mean (θ_0) and total sleep time for individual j (θ_1).

There are several mediated effects to examine. There is the mediated effect at level-1 (i.e., $\delta_1 \cdot \beta_1$), the mediated effect at level-2 (i.e., $\zeta_1 \cdot \gamma_1$), and the mediated effect at level-

3 (i.e., $\theta_1 \cdot \pi_1$). We are primarily interested in the level-1 mediated effect because this mediated effect indicates how total sleep time affected the number of spindles, which affected the KSS ratings within an individual within a given year. Thus, this is a within-person within-year mediated effect – essentially controlling for individual differences and maturational changes. Analyses that showed a significant within-subject, within-year spindle count effect were followed with a Sobel test [30] to determine if spindle count significantly mediated the TST effect.

Analyses for PVT LSNR, Sternberg slope, and Sternberg recency were similar to those described for KSS ratings. The analysis for the objective sleepiness with the MSLT was conducted using SAS PROC GLIMMIX to estimate a three-level discrete time survival model. Rather than determining the effect of one additional spindle, the spindle count measures were scaled so that they were the number of spindles produced by an additional hour of sleep (TST increases by approximately 1h for each 1.5 h step increase in TIB [14]). Central spindle count was scaled by dividing by 196, and frontal count was divided by 210.

We repeated all analyses substituting spindle density in the first 5 h of NREM sleep for spindle count.

To determine if significant spindle effects might be a proxy for non-specific light NREM sleep effects, we repeated the analyses replacing TST with N2 duration. We also evaluated whether objective daytime sleepiness was related to slow wave activity, the other hallmark of NREM sleep, by replacing spindle count with all night central delta (1-4 Hz) spectral energy.

Results

Effect of Time in Bed on Spindle Measures

All night frontal spindle count decreased significantly ($F_{2,144}$=111, p<0.0001) with decreasing TIB (Fig 1A). Mean spindle count for 7h TIB was 35% lower than that for 10h TIB. The spindle count for 7 h was lower ($F_{1,144}$=63.7, p<0.0001) than that for 8.5 h which was lower ($F_{1,144}$=33.2, p<0.0001) than that for 10 h TIB. Decreasing TIB was also significantly ($F_{2,144}$=97.4, p<0.0001) associated with a reduction in all night central spindle count (Figure 1A) with the mean count for 7 h TIB 32% smaller than that for 10h TIB. All night central spindle count for 7 h was smaller ($F_{1,144}$=53.1, p<0.0001) than that for 8.5 h which was smaller ($F_{1,144}$=31.2, p<0.0001) than that for 10 h TIB.

Reducing TIB not only decreased the total number of spindles but also decreased the rate of frontal spindle production ($F_{2,144}$=25.4, p<0.0001), i.e. spindle density per minute, in the first 5 hours of NREM sleep (Fig 1B). Mean frontal spindle density was 17% lower for 7h than for 10h TIB. Frontal spindle density for 7h TIB was significantly lower ($F_{1,144}$=18.8, p<0.0001) than that for 8.5h, and density for 8.5h was significantly lower ($F_{1,144}$=4.68, p=0.032) than for 10h. Central spindle density also decreased significantly ($F_{2,144}$=14.4, p<0.0001) with TIB reduction, with the 7h average density being 12% smaller than the 10h average density (Fig. 1B). Central spindle density was lower ($F_{1,144}$=11.0, p=0.0011) for 7 than for 8.5h TIB but did not differ significantly ($F_{1,144}$=2.42, p=0.12) between 8.5 and 10h.

Mean frontal spindle peak-trough amplitude (Fig 1C) in the first 5 h of NREM sleep decreased slightly (3% smaller mean amplitude for 7 than for 10h) but significantly ($F_{2,144}$=5.00, p=0.0079) with TIB reduction. Only the 7h vs. 10h comparison was

significant. The TIB effects were similar for central spindle peak trough amplitude ($F_{2,144}$=4.10, p=0.019), with the mean amplitude for 7 h TIB again being about 3% lower than that for 10h (Fig 1C). A second measure of spindle amplitude, Hilbert amplitude (Fig 1D), revealed similar TIB effects with both frontal ($F_{2,144}$=8.34, p=0.0004) and central ($F_{2,144}$=4.93, p=0.0085) Hilbert amplitude showing small but significant TIB effects.

Frontal spindle duration in the first 5h of NREM (Fig 1E) decreased slightly (1% difference in mean duration between 7 and 10 h TIB) but significantly ($F_{2,144}$=5.17, p=0.0068) with decreasing TIB. Only the 7h vs. 10h TIB comparison was significant ($F_{1,144}$=10.1, p<0.0018). Central spindle duration (Fig 1E) in the first 5 hours of NREM did not differ by TIB ($F_{2,144}$=1.87, p=0.16).

TIB duration affected none of the spindle frequency measures. Central ($F_{2,144}$=0.79, p=0.46) and frontal ($F_{2,144}$=0.20, p=0.82) spindle wave frequency (Fig. 1F) did not change with TIB, nor did the central sigma peak frequency ($F_{2,144}$=1.39, p=0.25) or the frontal sigma peak frequency ($F_{2,144}$=0.99, p=0.37).

Analysis of TIB effects on spindle measures in the last 5h of NREM sleep produced results very similar to those for the first 5h. Incidence and amplitude decreased as TIB was reduced. Statistical analysis results are summarized in Tables 1 and 2.

Effect of Sleep Duration on Spindle Measures

As we have reported in other publications from this study [14], total sleep duration decreased in a nearly linear manner as TIB was reduced from 10 to 8.5 h and from 8.5 to 7 h. A mixed effects analysis of total sleep time effects on spindle measures, produced similar effects to those of the analysis of TIB effects. All night spindle count,

spindle density, and amplitude measures decreased with decreasing TST for both central and frontal spindles. Frontal but not central spindle duration decreased significantly with TST reduction, and none of the spindle frequency measures changed with TST. Statistical analysis results for TST effects are summarized in Tables 1 and 2.

Age Effects and TIB by Age Interaction

With age effects modeled as linear effects, frontal and central all night spindle count and 5h NREM spindle density decreased significantly with age as did amplitude and duration. Central spindle frequency and peak frequency increased with age as did frontal spindle frequency but not frontal peak frequency. The TIB by age interaction was not significant for any of the spindle measures. In other words, between ages 10 and 16 years we did not detect an age-related change in the TIB effect on any spindle measure. Statistical analysis results for age effects and age by TIB interaction are summarized in Tables 1 and 2.

Sleep Spindle Effects on Daytime Sleepiness, Vigilance, and Working Memory

As shown in Fig 2A, within subjects within a year, the likelihood of falling asleep during the MSLT increased significantly ($F_{1,21426}$=17.4, p<0.0001) with decreasing frontal spindle count. Compared to an increase of 210 spindles, a decrease of 210 spindles was associated with a 38% increase in sleep likelihood. Furthermore, the Sobel test of mediation showed that frontal spindle count significantly (Z=4.17, p<0.0001) mediated the TST effect on MSLT sleep likelihood. Frontal spindle count accounted for 16.2% of the TST effect (Fig 2C). All night central spindle count was also significantly ($F_{1,21425}$=11.9, p=0.0006) associated with MSLT sleep likelihood. Compared to an increase of 196 spindles, a decrease of 196 spindles was associated with a 28%

increase in the likelihood of falling asleep during the MSLT. Central spindle count significantly mediated (Z=3.45, p=0.0006) the TST effect, explaining 12.6% of the effect. Subjective sleepiness rated on the Karolinska Sleepiness Scale (Fig 3A) was related to frontal spindle count ($F_{1,1553}$=10.49, p=0.0012) but not central spindle count ($F_{1,1553}$=1.97, p=0.16). Despite large decreases in frontal spindle count producing only a small increase in KSS ratings, frontal spindles significantly mediated (Z=3.23, p=0.0012) the TST effect on KSS ratings, explaining 11.9% of this effect.

The relation of increasing PVT performance to increasing frontal spindle count ($F_{1,1535}$=3.73, p=0.054) was not quite at the 0.05 level of significance (Fig 3B). Similarly, the 28% of the TST effect explained by fontal spindle count also was not quite significant mediation (Z=1.93 p=0.054). Performance on the psychomotor vigilance test (Fig 3B) was not related to central spindle count ($F_{1,1531}$=0.56, p=0.45).

Analysis of the relation of Sternberg test performance to night-4 spindle count found no effect of frontal ($F_{1,658}$=0.02, p=0.84) or central ($F_{1,658}$=0.79, p=0.37) spindle count on the slope of the function that describes the relation between response speed and memory set size. Nor was within subject within year spindle count related to the difference in response time for recent and nonrecent negative probes (frontal, $F_{1,658}$=0.21, p=0.65; central, $F_{1,658}$=0.56, p=0.45). It should be noted that neither Sternberg slope ($F_{1,658}$=0.77, p=0.38) or recency ($F_{1,658}$=0.04, p=0.85) were significantly related to within subject changes in prior sleep duration.

Two daytime measures, objective daytime sleepiness and ability to resist proactive interference, showed significant between subject effects. Subjects with more spindles were more likely to fall asleep during the MSLT (frontal, $F_{1,21426}$=7.81, p=0.0052; central,

$F_{1,21425}$=7.70, p=0.0055). Subjects with a greater number of spindles showed significantly (frontal, $F_{1,658}$=4.04, p=0.045; central, $F_{1,658}$=10.3, p=0.0014) lower resistance to proactive interference, i.e. a greater slowing of response time when the probe was in the previous memory set. Between subjects, the number of central or frontal spindles was not significantly related (p>0.28 for all) to subjective sleepiness, sustained vigilance, or working memory scanning efficiency.

Additional Analyses of Relations to Sleepiness, Vigilance, and Working Memory

Analyses of the relation of spindle density in the first 5 hours of NREM sleep to daytime sleepiness, vigilance, and memory produced results similar to those for all night spindle count. An increased likelihood of falling asleep during the MSLT was associated with both decreasing frontal ($F_{1,21425}$=23.8, p<0.0001) and central ($F_{1,21425}$=14.1, p=0.0002) spindle density. Furthermore, increased subjective sleepiness ratings on the KSS was associated with both decreasing frontal ($F_{1,1553}$=24.5, p<0.0001) and central ($F_{1,1553}$=14.7, p=0.0001) spindle density. Decreased vigilance on the PVT was associated with decreased frontal ($F_{1,1553}$=4.07, p=0.044) but not central ($F_{1,1537}$=1.02, p=0.31) spindle density, and neither frontal nor central spindle density was associated with either Sternberg outcome measure (p>0.24 for all).

In analyses that accounted for the effect of night 4 N2 duration rather than TST, likelihood of falling asleep during the MSLT still decreased significantly with increasing frontal ($F_{1,21426}$=7.69, p=0.0056) or central ($F_{1,21424}$=5.01, p=0.025) night 4 spindle count. Similarly, the frontal spindle effect on KSS subjective sleepiness ratings remained significant ($F_{1,1553}$=9.86, p=0.0017) when N2 duration replaced TST. The frontal spindle effect on the PVT was farther away from the alpha=0.05 threshold when accounting for

N2 duration ($F_{1,1535}$=3.55, p=0.060). Within subject within year, likelihood of falling asleep during the MSLT was not significantly related to all night delta energy ($F_{1,21425}$=0.29, p=0.59) when accounting for TST effects.

Discussion

Sleep restriction via shortening time in bed from 10 to 8.5 to 7 h greatly reduced total spindle count and spindle incidence but produced little to no change in other spindle measures. We interpret these sleep restriction effects as changes in sleep depth affecting the thalamocortical circuits that generate sleep spindles. The reduction in spindle count was associated with an increase in daytime sleepiness but no decrement in measures of memory and cognition.

Time in Bed and Sleep Duration Effects on Spindle Measures

As we previously documented [14], altering time in bed produced a nearly linear change in total sleep duration with TST decreasing from a mean of 530 min at 10h in bed to 406 min at 7h. However, the decrease in spindle count was not simply a result of decreased sleep duration. The approximately 23% TST decrease was associated with even larger decreases in frontal (35% decrease) and central (32% decrease) all night spindle count. Decreasing sleep duration preferentially reduces stage 2 sleep where spindles are more prevalent. Furthermore, limiting analysis to the first 5h of NREM sleep revealed that sleep restriction reduced the rate of spindle production. The reduced rate of spindle production likely resulted from a deeper sleep depth on the nights with 7h TIB. Both spindles and delta waves are generated in the same thalamocortical circuits [31]. Delta waves are produced when the cell membrane potential is more hyperpolarized, and

spindles are produced when the hyperpolarization is reduced. The 7h TIB condition that reduced the rate of spindle production also resulted in a small but significant increase in delta power [14]. Spindles have been proposed as a mechanism by which the brain protects the sleep state during lighter stages [10,32,33]. Spindle generation in thalamocortical circuits prevents the passage of arousing sensory information through the thalamus to the cortex [10]. Increased spindle production during the lighter sleep in the 10h TIB condition would decrease the likelihood of waking.

Spindle incidence is circadianly modulated [34]. Our protocol altered TIB by adjusting the bedtime not the risetime; therefore, for the three TIB conditions, the first 5 hours of NREM occur at different points in the 24 hour cycle. However, circadian differences cannot explain the TIB effects on spindle density which also increased with TIB for the last 5 hours of NREM sleep, the same circadian time period for all 3 TIB conditions.

TIB restriction was associated with only small changes in frontal and central spindle amplitude and frontal duration and was not associated with central spindle duration. The largest of these effects were 3% decreases in both frontal and central spindle amplitude. Our findings differ from those of Reynolds et al [18] who found that sleep restriction reduced amplitude and duration but not incidence. The studies differ in their TIB doses (Reynolds, 5, 7.5, and 10 h) and in their design. Reynolds et al noted that the within subject dose design used in our study would be an improvement over their own between subject study. We are confident that our 448 nights of EEG recording from 77 subjects provide an accurate representation of the effect of sleep restriction on sleep spindles in adolescents. Our finding of decreased density agrees with the finding of decreased density in the sleep following total sleep deprivation in young adults[17]

The frequency of waves within a sleep spindle and the frequency at which sigma power peaks increase linearly across adolescence [7-9]. We previously hypothesized that this increase was related to a decrease in sleep depth and the degree of hyperpolarization of thalamocortical neurons [8]. We expected to find that the deeper sleep with 7h TIB would decrease the frequency of sleep spindle waves compared to 10h TIB. Instead, TIB did not significantly affect frontal or central spindle frequency. Thus, decreasing sleep depth may not be the main reason that spindle wave frequency increases across adolescence. Tarokh et al [35] proposed that the adolescent increases in myelination could explain the rise in spindle frequency.

We have previously raised the possibility that sleep restriction might more strongly impact the EEG of younger adolescents. However, our current findings of no significant age-related change in the TIB effects on spindle measures do not support this hypothesis. These findings agree with our prior findings [14] that sleep restriction effects on delta, alpha, and sigma power also did not change across the 10 to 16 year age range of this study.

Sleep Spindles and Daytime Sleepiness and Vigilance

Delta (1-4 Hz) EEG activity, as expected for a marker of sleep dependent recuperative processes [36,37], is highest at the beginning of the night when the need for recuperation is greatest and decreases across the night as recuperation proceeds. As such, the sleep period can be shortened by 3 hours with little to no effect on the total amount of delta energy accumulated. Indeed, in the current dataset, reducing time in bed from 10 to 7 hours did not significantly decrease total delta energy [14]. We show here that all night delta energy was not significantly related to the daytime sleepiness that follows

nights of sleep restriction. Instead, the relation between MSLT sleep likelihood and night 4 spindle count, indicate that, a decrease in sleep spindles at least partially mediates the increase in sleepiness associated with reduction of prior sleep duration. Frontal spindles may be particularly critical for reduction of daytime sleepiness because they were associated not only with objective sleepiness measured with the MSLT but also with subjective sleepiness measured with the KSS. The persistence of significant relations between spindles and reduced sleepiness even when accounting for stage N2 duration indicate that sleep spindles themselves and not just the lighter phase of NREM sleep contribute to reducing daytime sleepiness. The relation between sleepiness measures and decreased spindle density in the first 5 hours of NREM sleep further indicates that the spindle decline is mediating a sleep loss effect and is not simply a result of a shorter period of recording. Spindles are clearly not the entire picture as they explain only a portion of the sleep duration effect on daytime sleepiness. Future studies could evaluate the contribution of other EEG markers such as K-complexes as well as non-EEG aspects of sleep.

Frontal spindles also showed a trend ($p=0.054$) toward an association with PVT performance. A study with a small number (n=8) of obstructive sleep apnea patients found that frontal but not central spindle count was related to faster PVT mean reaction time [38]. Spindles, particularly frontal spindles, may play a role in recuperative processes that reduce daytime sleepiness and restore the ability to maintain vigilance.

Sleep Spindles and Working Memory

We found no significant relation between sleep spindle count on night 4 and working memory scanning efficiency assessed with the Sternberg task on the next day. This

finding appears to conflict with previous studies showing a correlation between sleep spindle activity and performance on subsequent memory tasks. In young adults, a nap prevented degradation in performance on a face-name encoding test of episodic memory, and post-nap performance was correlated with frontal fast (but not slow) spindles [12]. Young adult declarative learning measured on a word encoding task improved following a nap, and the degree of improvement was correlated with central spindle count during the nap [13]. The authors proposed that transfer of information from the hippocampus to the cortex during the nap freed up hippocampal capacity for learning. Differences from our negative findings may be related to the particular memory test, declarative or episodic memory versus a working memory test that was not related to prior sleep duration. We also found no spindle-related between subject difference in working memory scanning efficiency. The only between subject difference in cognitive performance that we detected was a negative relation between sleep spindle count, both frontal and central, and ability to resist proactive interference. We note that the evidence thus far for a role for spindles in preparing the brain for subsequent learning is not strong. Additional studies that control for sleep duration are needed to firmly determine if spindles affect subsequent learning, which spindle property (amplitude, incidence, total count) is critical, and which, if any, type of learning or memory is affected.

Statistical Analysis Methods

We provided detailed descriptions of the multilevel statistical analyses used in this study because we believe that the study design and statistical analyses are an effective approach to evaluating the relation of sleep spindles to various outcome measures. A

within subject sleep restriction design will reduce sleep spindles. The multilevel analyses used here can assess the relation of spindle number to outcome measures such as a decrement in cognitive performance while accounting for non-spindle effects of sleep restriction such as decreased sleep duration. The analyses evaluate both the within subject effect of spindle reduction and between subject differences in spindle count. Our analyses include a third level for the within subject changes across the three years of the study.

Strengths and Weaknesses

The within subject dose response design with a large number of subjects allowed us to detect effects of sleep restriction that may have been obscured by between subject variability in sleep spindle measures. Furthermore, the relations of sleep spindle effects on daytime sleepiness, vigilance, and cognitive measures were evaluated with statistical analyses that teased out the spindle effects from nonspecific sleep duration effects. Weaknesses include the limited age range, 10 to 16 years, that may have been insufficient to detect age related changes in the effect of sleep restriction on sleep spindles. As we have noted previously [16], the 3 Hz window used during spindle detection is wider than the frequency difference between slow and fast spindles. We likely included some slow spindles in our central recordings and fast spindles in our frontal recordings.

Conclusion

This large-scale within subject study establishes that sleep restriction profoundly decreases spindle count and the rate of spindle production. Spindle reduction via time in bed restriction produces increases in objective and subjective daytime sleepiness that

can be statistically distinguished from the effect of sleep loss in general. We have provided detailed descriptions of the statistical methods used because the methods used here are an effective approach to test relations between sleep spindles and daytime cognitive measures.

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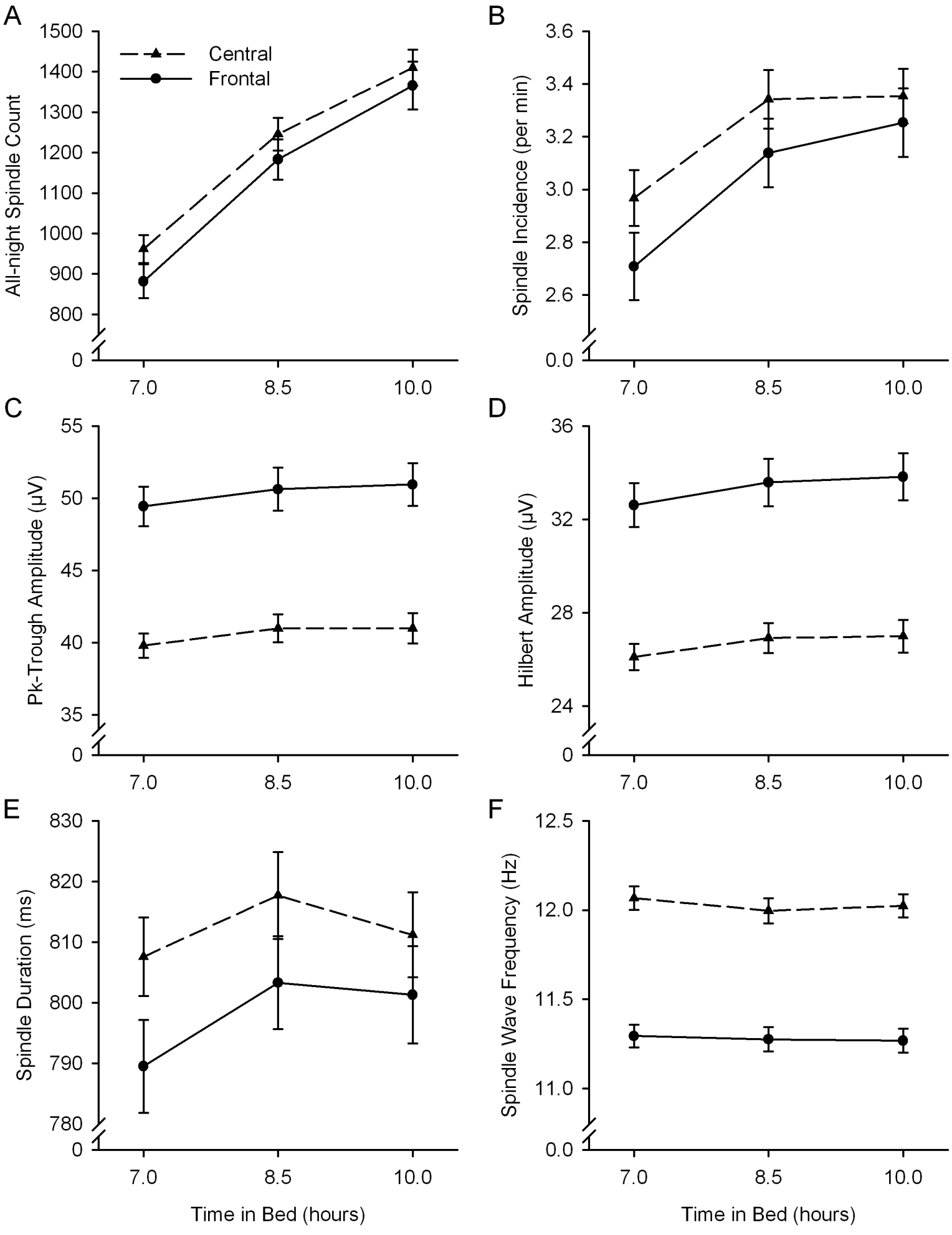
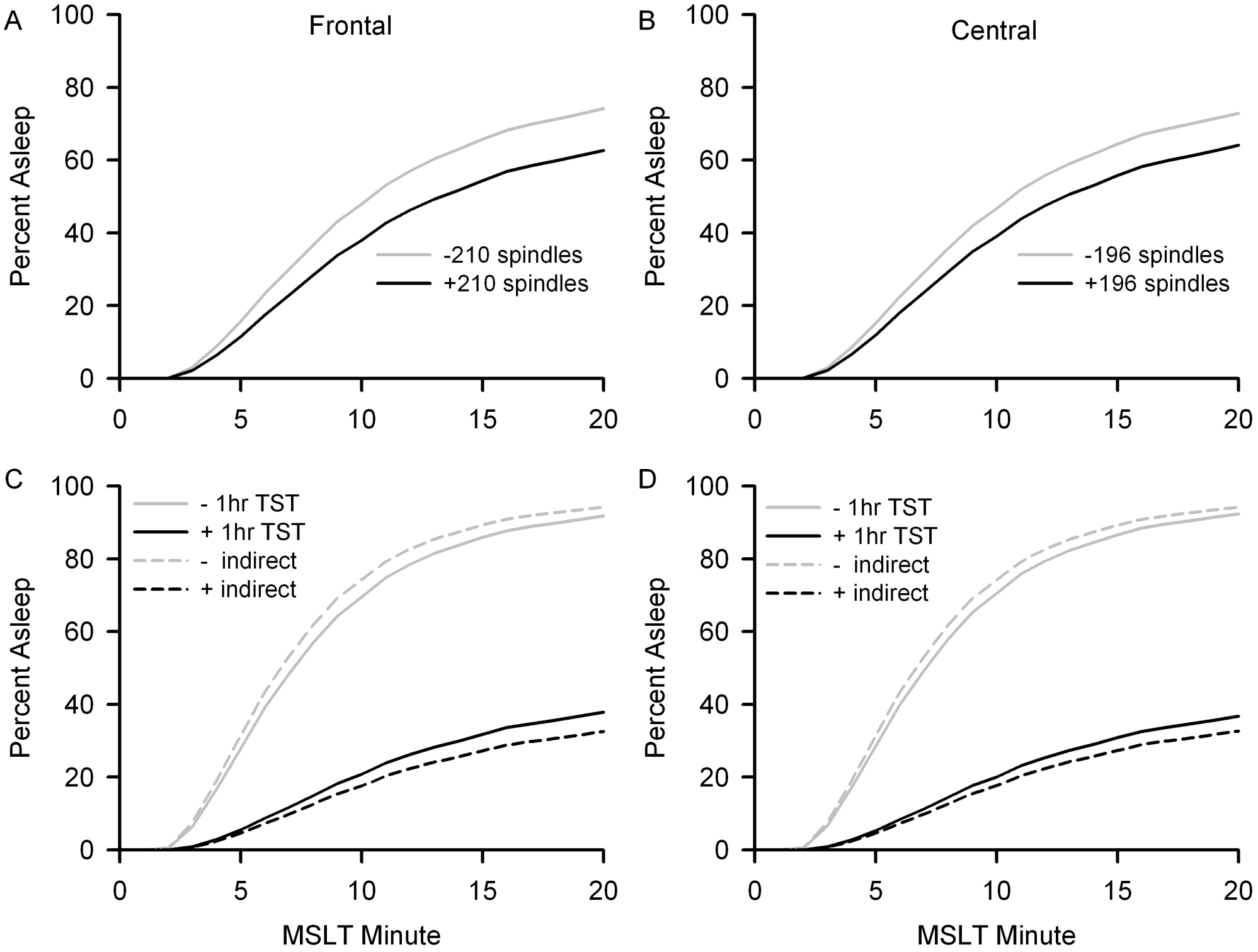
Figure Captions

Figure 1. For frontal (circles, solid line) and central (triangles, dashed line) spindles, mean +/- standard error is plotted against time in bed (TIB) for the following measures: (A) all-night count, (B) incidence, (C) peak trough amplitude, (D) Hilbert amplitude, (E) duration, and (F) wave frequency. TIB effects were largest for spindle count and incidence, but changing TIB also produced small but significant effects on amplitude.

Figure 2. Multilevel model estimated percentage of subjects asleep is plotted against minute of the multiple sleep latency test (MSLT). Likelihood of falling asleep increased with decreasing frontal (A) and central (B) spindle count as shown by the greater percent asleep for the gray line representing the decrease in spindle count associated with one less hour of total sleep time (TST) compared to the black line representing spindle count associated with an additional hour of TST. Spindle count significantly mediated the TST effect on MSLT sleep likelihood with the indirect effect accounting for 16.2% of the TST effect for frontal spindles (C) and 12.6% for central spindles (D).

Figure 3: Multilevel model estimates of the relation between daytime sleepiness or cognition measures and frontal (solid line) or central (dashed line) all night spindle count. (A) Subjective sleepiness rating on the Karolinska Sleepiness Scale (KSS) was significantly related to frontal but not central spindle count. (B) The relation between sustained vigilance measured on the psychomotor vigilance test (PVT) and spindle count approached significance for frontal but not central spindles. (C) Working memory scanning efficiency measured with the Sternberg test was not associated with spindle count nor was the ability to resist proactive interference (D).


A
All-night Spindle Count
1500
1400
1300
1200
1100
1000
900
800
0
Central
Frontal
7.0
8.5
10.0
B
Spindle Incidence (per min)
3.6
3.4
3.2
3.0
2.8
2.6
0.0
7.0
8.5
10.0
C
Pk-Trough Amplitude (μV)
55
50
45
40
35
0
7.0
8.5
10.0
D
Hilbert Amplitude (μV)
36
32
28
24
0
7.0
8.5
10.0
E
Spindle Duration (ms)
830
820
810
800
790
780
0
7.0
8.5
10.0
Time in Bed (hours)
F
Spindle Wave Frequency (Hz)
12.5
12.0
11.5
11.0
0.0
7.0
8.5
10.0
Time in Bed (hours)


A
Frontal
Percent Asleep
100
80
60
40
20
0
0
5
10
15
20
-210 spindles
+210 spindles
B
Central
Percent Asleep
-196 spindles
+196 spindles
C
- 1hr TST
+ 1hr TST
- indirect
+ indirect
Percent Asleep
MSLT Minute
D
- 1hr TST
+ 1hr TST
- indirect
+ indirect
Percent Asleep
MSLT Minute

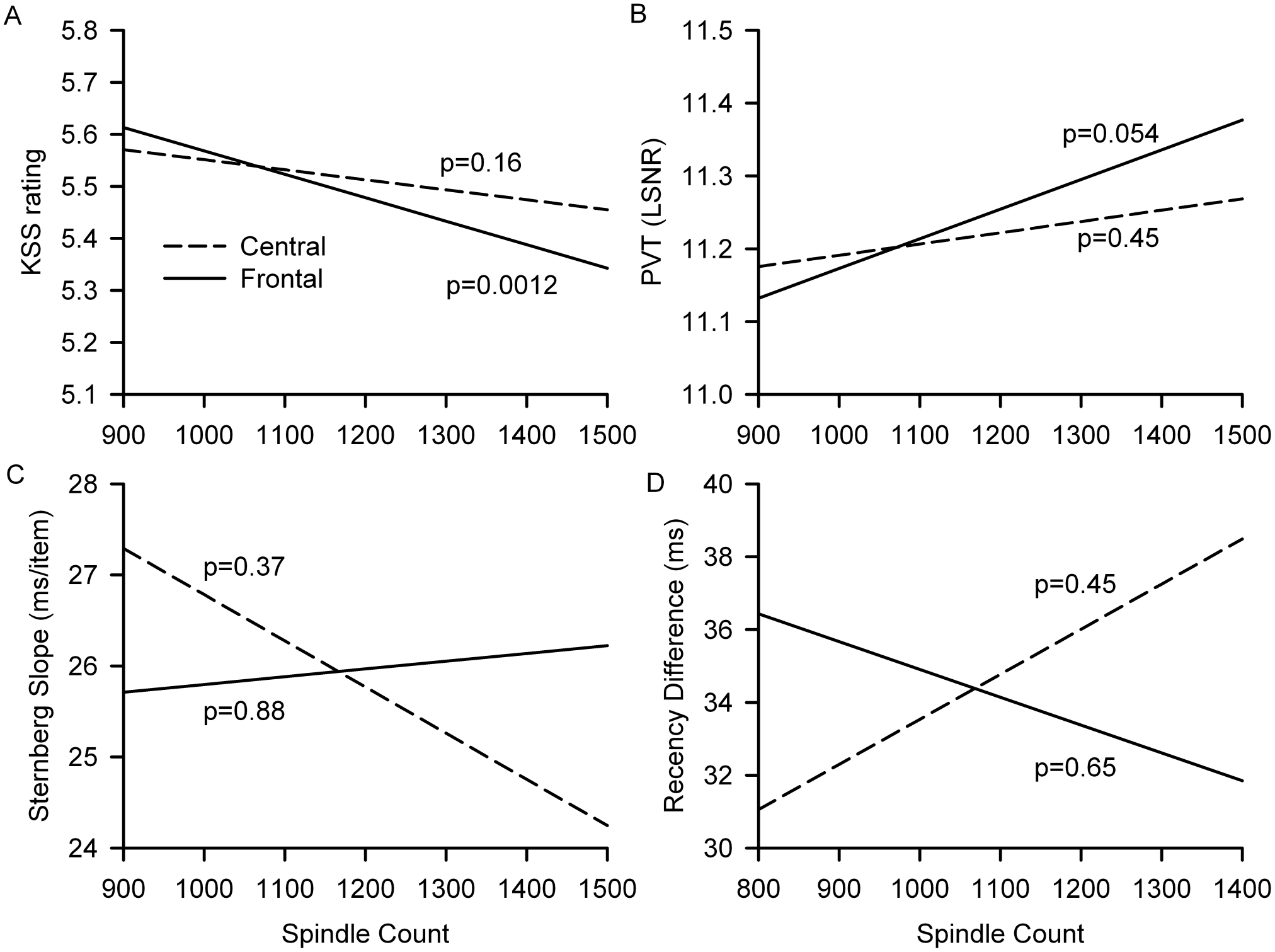

A
KSS rating
5.8
5.7
5.6
5.5
5.4
5.3
5.2
5.1
p=0.16
Central
Frontal
p=0.0012
900
1000
1100
1200
1300
1400
1500
B
PVT (LSNR)
11.5
11.4
11.3
11.2
11.1
11.0
p=0.054
p=0.45
C
Sternberg Slope (ms/item)
28
27
26
25
24
p=0.37
p=0.88
Spindle Count
D
Recency Difference (ms)
40
38
36
34
32
30
p=0.45
p=0.65
800
Spindle Count

Table 1. Results, p values, of mixed effects analyses of time in bed (TIB) and age effects on frontal spindle measures.

Spindle Measure	TIB	10h vs. 7h	10h vs. 8.5h	8.5h vs. 7h	Age	TIB x Age	TST
All night count	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.92	**<0.0001**
Density 5h	**<0.0001**	**<0.0001**	**0.032**	**<0.0001**	**0.0004**	0.99	**<0.0001**
Pk-Tr Amp 5h	**0.0079**	**0.0019**	0.18	0.12	**0.0036**	0.87	**<0.0001**
Hilbert Amp 5h	**0.0004**	**<0.0001**	0.11	**0.035**	**0.014**	0.70	**<0.0001**
Duration 5h	**0.0068**	**0.0018**	0.26	0.070	**<0.0001**	0.69	**0.0035**
Frequency 5h	0.82				**0.0029**	0.26	0.20
Density L5h	**<0.0001**	**<0.0001**	**<.0001**	**<0.0001**	**<0.0001**	0.87	**<0.0001**
Pk-Tr Amp L5h	**0.0079**	**0.0077**	0.27	0.17	**0.0031**	0.84	**0.0004**
Hilbert Amp L5h	**0.0014**	**0.0003**	0.16	**0.049**	**0.013**	0.68	**<0.0001**
Duration L5h	**0.015**	**0.0045**	0.38	0.076	**<0.0001**	0.74	**0.0070**
Frequency L5h	0.80				**0.015**	0.19	0.95
Peak Freq	0.37				0.78	0.20	0.54

For measures with significant TIB effects, results of multiple comparisons are also shown. The right hand column shows results of analyses with total sleep time replacing TIB. Significant ($p<0.05$) results are indicated by bold font. 5h indicates measures for the first 5h of NREM sleep. L5h indicates measures for the last 5h of NREM sleep.

Table 2. Results, p values, of mixed effects analyses of time in bed (TIB) and age effects on central spindle measures.

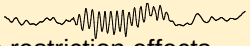
Spindle Measure	TIB	10h vs. 7h	10h vs. 8.5h	8.5h vs. 7h	Age	TIB x Age	TST
All night count	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.11	**<0.0001**
Density 5h	**<0.0001**	**<0.0001**	0.12	**0.0011**	**0.0001**	0.27	**<0.0001**
Pk-Tr Amp 5h	**0.019**	**0.0058**	0.065	0.48	**<0.0001**	0.095	**0.0016**
Hilbert Amp 5h	**0.0085**	**0.0025**	**0.046**	0.42	**<0.0001**	0.091	**0.0005**
Duration 5h	0.16				**<0.0001**	0.87	0.081
Frequency 5h	0.46				**<0.0001**	0.20	0.24
Density L5h	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.12	**<0.0001**
Pk-Tr Amp L5h	**0.030**	**0.010**	0.078	0.54	**<0.0001**	0.095	**0.0032**
Hilbert Amp L5h	**0.018**	**0.0056**	0.064	0.48	**<0.0001**	0.083	**0.0013**
Duration L5h	0.27				**<0.0001**	0.78	0.16
Frequency L5h	0.78				**<0.0001**	0.28	0.79
Peak Freq	0.25				**<0.0001**	0.54	0.14

For measures with significant TIB effects, results of multiple comparisons are also shown. The right hand column shows results of analyses with total sleep time replacing TIB. Significant ($p<0.05$) results are indicated by bold font. 5h indicates measures for the first 5h of NREM sleep. L5h indicates measures for the last 5h of NREM sleep.

Sleep restriction effects on sleep spindles in adolescents and relation of these effects to subsequent daytime sleepiness and cognition

Questions:

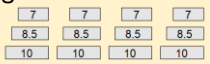
1. How does sleep restriction affect sleep spindles in adolescents?



2. Do these sleep restriction effects change with age?
3. Are sleep restriction effects on spindles related to changes in daytime sleepiness and cognitive performance?

Intervention:

- Annually for 3 years, participants kept each of thee sleep schedules: 7, 8.5, and 10 hours in bed for 4 nights consecutively.

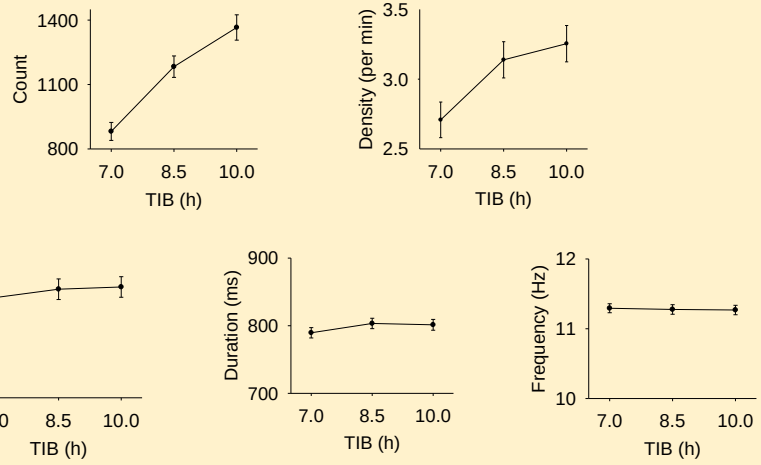


Measures:

- Automated sleep spindle detection in frontal and central EEG recorded on night 4.
- Daytime objective (MSLT) and self-reported (KSS) sleepiness.
- Daytime vigilance (PVT) and working memory (Sternberg).

Findings:

1. Reducing time in bed (TIB) decreased spindle count and spindle density and had small or no effect on spindle amplitude, duration, and frequency



2. Sleep restriction effects on spindles did not change with age.

3. In analysis that accounted for the effect of sleep duration, decreased spindle count was associated with increased MSLT sleep likelihood.

   Spindle count significantly mediated the sleep duration effect on sleepiness, explaining 16% of the sleep duration effect.

   Similar analyses found no relation between spindle count and subsequent working memory scanning efficiency or resistance to proactive interference.