

Correspondence

Spontaneous brain rhythms predict sleep stability in the face of noise

Thien Thanh Dang-Vu^{1,3}, Scott M. McKinney², Orfeu M. Buxton^{1,4}, Jo M. Solet^{1,5} and Jeffrey M. Ellenbogen^{1,2}

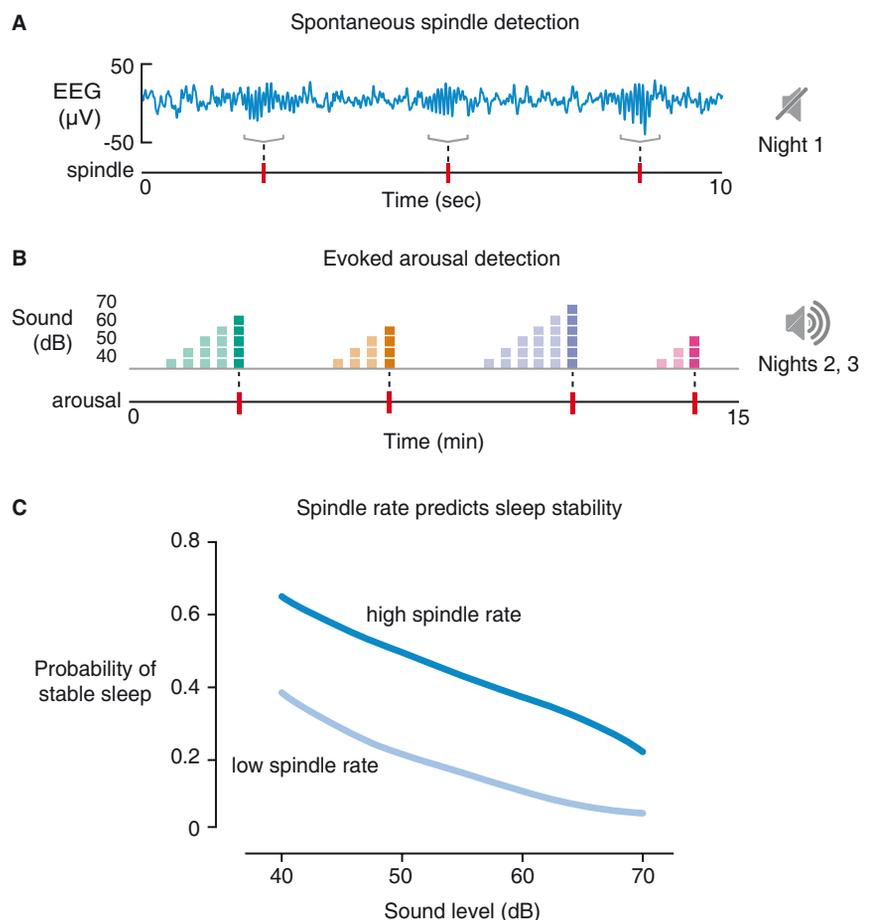
Quality sleep is an essential part of health and well-being. Yet fractured sleep is disturbingly prevalent in our society, partly due to insults from a variety of noises [1]. Common experience suggests that this fragility of sleep is highly variable between people, but it is unclear what mechanisms drive these differences. Here we show that it is possible to predict an individual's ability to maintain sleep in the face of sound using spontaneous brain rhythms from electroencephalography (EEG). The sleep spindle is a thalamocortical rhythm manifested on the EEG as a brief 11–15 Hz oscillation and is thought to be capable of modulating the influence of external stimuli [2]. Its rate of occurrence, while variable across people, is stable across nights [3]. We found that individuals who generated more sleep spindles during a quiet night of sleep went on to exhibit higher tolerance for noise during a subsequent, noisy night of sleep. This result shows that the sleeping brain's spontaneous activity heralds individual resilience to disruptive stimuli. Our finding sets the stage for future studies that attempt to augment spindle production to enhance sleep continuity when confronted with noise.

The brain's response to sensory input is modulated by ongoing, spontaneous neuronal activity [4]. Indeed, during sleep, the thalamus spontaneously engages with the cortex. This interaction can produce transient fluctuations of the brain's electric field visible on the EEG as rhythmic spindles (Figure 1A). As the thalamus relays sensory information to perceptual cortices, it has been proposed that brain processes involved in spindle production gate sensory input during sleep [2]. If spindles hinder the transmission of external stimuli from the thalamus to

the cortex, a higher rate of spindle production throughout the night would be expected to preserve sleep stability in the face of noise. We hypothesized that individuals who generate more spindles would require sounds of higher intensity to disrupt their naturally occurring sleep.

Twelve healthy human volunteers (age 26.3 ± 7.5 , mean \pm SD) were studied in the sleep laboratory for three consecutive nights. The first night was quiet, while the second and third were noisy. Brain activity was monitored each night with EEG. We detected spindles on central channels (C3, C4) during the quiet night using an automatic algorithm (Figure 1A, and

Figure S1A in the on-line Supplemental Information), defining each subject's spindle rate as the number of detected events per minute during stage N2 and N3 (stages 2 and 3 of non-REM sleep). On the noisy nights we presented frequently encountered sounds — for example road and air traffic, a telephone ringing, or hospital-based mechanical sounds — during stages N2, N3 and R (REM sleep). These ten-second noises were initiated at 40 decibels (dB) and presented every thirty seconds in 5 dB increments until the EEG signal was perturbed according to standard guidelines (that is, an arousal was observed) [5] (Figure 1B). In the present analysis, sleep



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Figure 1. Spindle rate predicts sleep stability.

(A) Sleep spindles were automatically detected on central EEG channels during a quiet night of sleep. The number of detected events (vertical bars on the bottom line) per minute defined each subject's spindle rate. (B) On two subsequent nights we introduced ten-second noises, initiated at 40 decibels (dB) and presented every thirty seconds in 5 dB increments until the EEG signal was perturbed (arousal, vertical bars on the bottom line). Each colour represents a different sound type; a sample of four is shown here. (C) Observations were pooled among subjects in the lower and upper halves of the spindle rate distribution (ranges 4.57–5.44 and 5.48–6.14 spindles/min, respectively) based on EEG lead C3 during stage N2. Corresponding sleep survival curves were derived from each pool in stage N2 using the Kaplan-Meier (product-limit) method.

stability is defined as the maintenance of sleep without arousal.

We first considered the relationship between spindles and sleep stability during stage N2, when spindles predominate. Using Cox regression, we found that those with higher spindle rates on the quiet night exhibited greater sleep stability during the noisy nights: spindle rate carried a sleep disruption hazard ratio (HR) of 0.39 from C3 ($p = 0.001$) and 0.51 from C4 ($p = 0.002$) (see Figure 1C).

As spindles are also present in stage N3, we performed the same analysis considering stages N2 and N3 together. We again found a significant relationship between spindle rate and sleep stability (HR = 0.55, $p = 0.003$ for C3; HR = 0.64, $p = 0.018$ for C4).

This result shows that it is possible to predict an individual's ability to maintain sleep in the face of external sound: those with more abundant spindles are more resistant to sounds during sleep. It remains to be seen whether this relationship emerges from the cumulative effects of spindle and sound collision, as we suspect, or whether it is due to a yet undetermined biological process.

In line with previous reports [3], we observed consistent spindle rates from night to night (Figure S1B). We thus regard spindle rate as a stable trait, suitable for predicting sleep continuity under noisy conditions.

The extent to which the relationship between spindle rate and noise tolerance bears on different populations awaits exploration. Noise tolerance during sleep [6], like spindle rate [7], diminishes with age. On the other hand, despite reporting poor sleep, people with insomnia possess arousal thresholds similar to those of normal sleepers [8]. They likewise produce spindles at normal rates [9]. It is tempting to link these pairs of observations based on our result.

Our finding also suggests a tantalizing explanation for associations uncovered between spindle rate and learning potential (see for instance [10]): in addition to perhaps actively contributing to memory consolidation, spindles may shield sleep from disruption, allowing consolidating processes to operate unhindered.

Our data raise important questions about whether augmenting spindle rate through behaviour, drug or device might protect sleep by harnessing the spindle's ability to deflect incoming

stimuli. While we await intervention-based exploration, this study provides evidence that sleep spindle rate — readily quantified from EEG — serves as a biomarker for vulnerability to sound during sleep.

Supplemental Information

Supplemental Information is available at doi:10.1016/j.cub.2010.06.032

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Supplemental Data: Spontaneous brain rhythms predict sleep stability in the face of noise

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Supplemental Experimental Procedures

Institutional review

Study procedures were approved by the Human Research Committees of the Brigham and Women's Hospital, Massachusetts General Hospital (MGH) and the Cambridge Health Alliance. Informed consent was obtained for all subjects.

Subjects

Twelve healthy volunteers (8 females and 4 males, age 26.3 ± 7.5 [mean \pm SD]) were screened to be free from any acute or chronic medical or psychiatric conditions, established on the basis of clinical history and a physical examination obtained by a physician. History of illicit drug, alcohol, or caffeine dependency was also ruled out by clinical history and confirmed by toxicological urinalysis. Participants were taking no medications (prescription or over the counter) that affect circadian rhythms or sleep. Absence of sleep pathologies, including sleep-disordered breathing and periodic limb movements, was confirmed by standard polysomnographic sleep recording (see Sleep recordings). Volunteers with hearing impairment were excluded based on audiometric screening of each ear (hearing level threshold of 25 decibels [dB] at 500, 1000, 2000 and 4000Hz).

Study conditions

Prior to the study, subjects slept at home on a consistent schedule for at least 4 days, as assessed by wrist actigraphy (AW-64, Minimitter, Bend OR). Participants were admitted to the MGH Sleep Laboratory in the early evening and stayed for 3 days. Subjects were given

the opportunity to sleep for 8.5 hours at their normal bedtime. Research staff was present 24 hours a day to monitor the subject via audio and video, as well as to check vital signs, deliver meals, and ensure that subjects did not nap. Light levels were maintained at approximately 90 lux during waking periods, and <1 lux during sleep periods in order to maintain normal circadian alignment.

Sleep recordings

Polysomnographic (PSG) recordings were collected using a Comet XL system (Grass-Telefactor West Warwick, RI, USA). On all three nights, skin surface electrodes (Beckman Instrument Company, Schiller Park, IL) captured EEG from frontal (F3 and F4), central (C3 and C4) and occipital (O1 and O2) positions, electrooculogram (EOG), submental electromyogram (EMG), and electrocardiogram (ECG). Data were captured at a sampling rate of 200 Hz and conditioned by analog filters (high pass 0.3 Hz, low pass 70 Hz).

Acoustic stimuli

Fourteen sounds were recorded in a medical unit of Somerville Hospital, Somerville, Massachusetts. Sounds were drawn from a broad range of sources: an IV alarm, a phone ringing, a toilet flush, a physician's pager sounding, a door creaking and slamming, a laundry machine, an ice machine, a towel dispenser, traffic noises, snoring, a jet engine, a helicopter, and two conversations of different emotional valence (positive and negative). All sounds were 10 seconds in duration.

Sounds were presented during sleep on the second and third nights of the study. The first night was quiet, containing merely "sham" acoustic stimulation: the audio equipment was positioned as it would be on subsequent (noisy) nights, but sleep was not disturbed for the

entire 8.5 hr sleep opportunity. Subjects were not informed that they would have an initial quiet night, but were rather told that sounds would be delivered during all nights.

On the noisy nights, acoustic stimuli were presented in surround sound using an array of four studio-monitor loudspeakers (Event, model PS6) placed at the circumference of a virtual circle around the subject's head (i.e., modified ITU-R BS775-1 pattern). All sounds were veridical with respect to the soundscape. If a sound in real life had motion, such as a plane or car, then it was played as having motion in space during the study. The mean (\pm SE) number of presented sounds per subject was 193.1 (19.6) for the second night and 242.5 (34) for the third night. Stimuli were presented on a measured average background of 34-35 dB that was due to continuous ventilation in the room.

Sound levels were measured using dBA- $L_{\text{eq-10sec}}$, consistent with standard methods used to evaluate the clinical effects of noise: 'A' refers to the weighting of sounds in ranges audible to humans; $L_{\text{eq-10sec}}$ refers to the equivalent continuous sound level, i.e., averaging of sound pressure level across the 10 sec of sound presentation.

The sound level in the patient room was logged with an environmental sound monitor (Rion Type NL-31, with Type 1 microphone) installed on a tripod roughly 10 inches above the head of the sleeping subject, and programmed to output a DC voltage proportional to the A-weighted fast response sound level. This signal was integrated by the sleep recording software and calibrated using a 1 kHz sine wave.

Once stable sleep was achieved (at least 90 seconds of the same stage was scored in real time), sounds were initiated at 40 dB and presented every thirty seconds in 5 dB increments

until an arousal was observed or until 70 dB was reached. A 70 dB limit was set in order to minimize full awakenings from sleep and thus prevent significant disruption of sleep architecture (Supplemental Results). Stimuli were presented in randomized order for each participant and night. Each time a sound elicited an arousal, no further sound was presented until stable sleep resumed.

Sleep analysis

Sleep stages, spindles and arousals were identified in adherence with the recommendations of the American Academy of Sleep Medicine [5]. According to these criteria, an arousal is defined as an abrupt increase in EEG frequency that lasts at least 3 seconds, excluding that caused by a spindle, preceded by at least 10 seconds of stable sleep. Scoring of sleep was conducted by a registered polysomnographic technician under the supervision of the medical director of the MGH sleep laboratory.

Arousal threshold

Acoustic arousal threshold was defined as the sound intensity observed to evoke an EEG arousal. The mean (\pm SE) number of sound-evoked arousals per subject was 42.7 (\pm 2.8) for the second night, and 44.6 (\pm 4.2) for the third night. Because our hypothesis focused on spindles, arousal thresholds were first examined in epochs of stage N2 sleep, the stage during which spindles predominate [5]. As spindles are also present during stage N3, sounds and corresponding arousal thresholds were also examined during stages N2 and N3 considered together. Sounds were delivered during two nights in order to increase the sample of presented sounds [S1].

For the computation of the linear regression (see Statistical analysis section), arousal thresholds were averaged across sound types within subject.

Spindle rate

Spindle rate was quantified on EEG channels C3 and C4 (referenced to the contralateral mastoid), since they are most pronounced in these locations [7]. To this end, an automatic spindle detection algorithm was adapted from Molle et al. and others [S2-S3] (Figure S1A). According to this method, the raw EEG signal was digitally bandpass filtered in the spindle frequency range (11-15 Hz) using a linear phase finite impulse response filters (-6 dB at 11 and 15 Hz). The average root mean square (rms) power of the filtered signal was calculated in time windows of 0.25 sec with 5 msec resolution. Sleep spindles were identified during those times in which the rms power of the filtered signal achieved a value above its 87th percentile. The presence of a spindle was validated by determining whether the spindle's peak-to-peak amplitude lay between 10 and 100 μ V, and its duration was at least 0.5 sec. Spindle rate was calculated as the ratio of the number of detected spindles during stages N2 and N3 to the duration (in min.) of these stages in each subject.

Because of arguments suggesting the existence of two types of spindles (fast and slow) [S3], we also tested whether there was an effect of spindle type on sleep stability in the face of noise (Supplemental Results). Detected spindles were thus classified as either fast or slow according to the peak frequency of the filtered EEG signal during each detected spindle (slow = 11-13 Hz; fast = 13-15 Hz).

In order to test whether other properties of spindles, in addition to their rate, modulate sleep stability in the face of noise, we computed the average amplitude and duration of spindles

characteristic to each subject. As described above, the beginning and end of a spindle were defined as two successive threshold-crossings of the power of the filtered signal, at least 0.5 sec apart. The amplitude was computed as the maximal peak-to-peak amplitude of the filtered EEG signal during a detected spindle. We also tested a joint measure of amplitude and duration by computing the total spectral power of the filtered signal (11-15 Hz) during detected spindles (Supplemental Results). Power spectra were estimated using the multitaper method [S4].

We confirmed that spindle rate is consistent across nights, as shown previously [S5], by measuring spindle rates during the second and third (noisy) nights in addition to the first (quiet) night. To achieve this, we applied the spindle detection algorithm to EEG collected from the second and third nights, after excluding all epochs during which sounds were presented, as well as those immediately afterward. This exclusion was performed because sounds are known to influence spindle production [S6]. (See Supplemental Results below, and Figure S1B.)

Statistical analysis

Sleep stability was defined as the maintenance of sleep in the absence of arousal. Sleep stability was interrogated using survival analysis, where maintenance of sleep constitutes survival, and arousal from sleep a failure. Each sound series defined a distinct risk period during which sleep could be disrupted by a sound-induced arousal.

Empirical descriptions of the cumulative distribution of arousal thresholds, called survivor functions, were estimated using the Kaplan-Meier product-limit method for censored data. The survivor function of loudness describes the probability of tolerating sound intensities at

least that loud. Across all 565 observations, 138 were right-censored, meaning that sound presentation at the highest dB level was terminated before an arousal occurred.

The effect of spindle rates from EEG channels C3 and C4 on sleep stability (i.e., absence of arousal) was evaluated using a Cox proportional hazards regression model, accounting for shared frailty among sleep periods belonging to the same subject.

The hazard ratio (similar to a measure of relative risk) represents the proportional change in hazard due to a one-unit increase in spindle rate. In the current analysis, more spindles per minute resulted in a significantly lower hazard of sleep disruption due to noise. The same method was used to test the effects of spindle amplitude, duration and power on stage N2 sleep stability.

As an alternative presentation of these data, we also tested whether spindle rates were significantly correlated to each individual's mean arousal threshold using a linear regression. In this case, right-censored events were considered as arousal thresholds of 75 dB for the calculation of the mean. (Figure S1C and Supplemental Results, below.) This further substantiates our finding.

Supplemental Results

Sleep stages

Table S1 shows the composition of total sleep time (TST) averaged across subjects, during each of the three nights in this study. These values did not differ significantly across nights ($F = 0.00016$; $p = 0.99$), showing that, aside from very brief arousals, traditionally-defined sleep architecture was not affected by sound presentation. In agreement with previous reports [S7],

amounts of stages N1, N2, N3 and REM sleep (either absolute time or % of TST) and TST were not significantly correlated with arousal thresholds during stage N2.

We also compared the same parameters from the quiet night (amounts of stages N1, N2, N3 and REM, TST) among subjects in the upper and lower halves of the spindle rate distribution, as depicted in figure 1C, and found no significant difference (two-sample t-tests). This suggests that the effects observed with spindle rate are not confounded by sleep stages and total sleep time.

Spindle rate

Spindle rates on C3 were not significantly different from spindle rates on C4 (paired t-test). Spindle rates were not significantly different between females and males, either on C3 or C4 (two-sample t-test).

In order to establish that spindle rate was consistent night to night for a given subject, we compared spindle rate on the quiet night to spindle rate on the quiet portions of the noisy nights. Thus, spindle rates on nights 2 and 3 were calculated after exclusion of 30-sec epochs with sounds, as well as the subsequent epoch after sound presentation. In order to compensate for the fewer number of quiet epochs in nights 2 and 3 compared to night 1, spindle rates in nights 2 and 3 were considered together. Spindles rates were not significantly different across nights (ANOVA), either for C3 ($p = 0.15$), or C4 ($p = 0.43$). Spindle rates on night 1 were also positively correlated with those on nights 2 and 3 ($r = 0.63$, $p = 0.028$ for C4; $r = 0.68$, $p = 0.01$ for C3) (Figure S1B). This stands in agreement with previous reports, showing a consistency of spindle rate across nights within subjects [S5]. In addition, using spindle rates

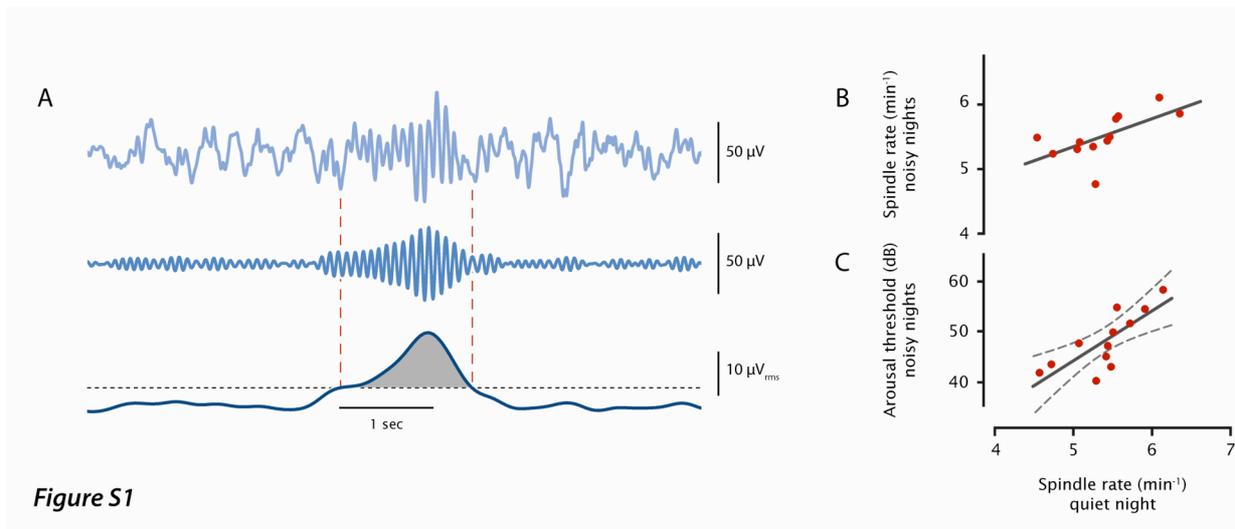
from nights 2 and 3 (instead of the first, quiet, night) still predicted sleep stability in the face of noise during the same nights (Cox regression: $p < 0.001$ for C3, $p = 0.028$ for C4).

The average amplitude, duration or power of spindles during the quiet night did not have a significant effect on sleep stability during noisy nights. The spindle rate weighted by either the mean amplitude or mean spectral power of spindles likewise did not predict sleep stability. Only the spindle rate weighted by duration achieved significance with sleep stability ($p = 0.013$ and $p = 0.012$, for C3 and C4, respectively). However, we found that spindle rate weighted by the spindles' average duration was strongly correlated with spindle rate alone ($R^2 = 0.845$), demonstrating that this effect was likely driven by spindle rate alone with no effect of duration itself. This was due to the relative consistency in spindle duration across subjects (mean duration = 0.84 sec; SD = 0.035 sec). When spindles were classified into fast and slow spindles, the corresponding slow and fast spindle rates had no significant effect on sleep stability from noise. Altogether, these additional analyses suggest that the number (rate) rather than the properties (amplitude, duration, power or frequency) of spindles modulates sleep stability in the face of noise.

Figure S1C shows the correlation during stage N2 between spindle rate and mean arousal threshold as assessed by linear regression. We found that spindle rate on a quiet night was positively correlated with arousal threshold during noisy nights ($r = 0.77$; $p = 0.003$ for C3; $r = 0.71$; $p = 0.01$ for C4).

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A. *Spindle detection method*

(Upper trace) Electroencephalogram (EEG) depicting a typical spindle. This raw trace was derived from lead C3, referenced to the contralateral mastoid.

(Middle trace) For spindle detection, the raw signal (shown in upper trace) was first filtered in the spindle frequency band (11-15 Hz).

(Lower trace) Sleep spindles were then identified during those times when the average root mean square (rms) power of the filtered signal (shown in middle trace) achieved a value above its 87th percentile (horizontal dashed line). Additional criteria (not shown) included peak-to-peak amplitude between 10 and 100 μV , and duration longer than 0.5 sec. The vertical dashed lines delineate the detected spindle.

B. *Spindle rate is consistent across nights*

Spindle rate during the first (quiet) night positively correlated with spindle rate averaged over the second and third (noisy) nights ($r = 0.63$; $p = 0.028$), after exclusion of 30 sec-epochs during which sounds were presented as well as the epoch immediately following. Spindles rates are from EEG lead C4 and in stage N2 sleep. Each dot represents one subject. The solid line is the regression.

C. Spindle rate predicts mean arousal threshold

Spindle rate during the quiet night positively correlated with mean arousal threshold during noisy nights ($r = 0.77$; $p = 0.003$; the spindle rates represented here are calculated from EEG lead C3) during stage N2 sleep. Each dot represents one subject. The solid line is the regression. The dashed lines represent the 95% confidence interval.

Table S1. Sleep composition across nights

Sleep stage	night 1 (quiet)	night 2 (noisy)	night 3 (noisy)
Wake	28±4	26±6	31±5
Stage N1	52±6	53±6	54±9
Stage N2	239±9	254±10	237±9
Stage N3	92±10	68±8	89±11
REM	97±6	107±5	99±6
Latency to N2	13±2	12±2	16±3
Latency to N3	26±3	24±2	26±4
Latency to REM	94±11	79±8	74±6
Total Sleep	481±4	482±6	479±5

Mean time (\pm SE; min.) spent in different stages of sleep, and their corresponding latencies, across the 3 experimental nights.