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SHORT PAPER

# Naps reliably estimate nocturnal sleep spindle density in health and schizophrenia

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## Abstract

Sleep spindles, defining oscillations of non-rapid eye movement stage 2 sleep (N2), mediate memory consolidation. Spindle density (spindles/minute) is a stable, heritable feature of the sleep electroencephalogram. In schizophrenia, reduced spindle density correlates with impaired sleep-dependent memory consolidation and is a promising treatment target. Measuring sleep spindles is also important for basic studies of memory. However, overnight sleep studies are expensive, time consuming and require considerable infrastructure. Here we investigated whether afternoon naps can reliably and accurately estimate nocturnal spindle density in health and schizophrenia. Fourteen schizophrenia patients and eight healthy controls had polysomnography during two overnights and three afternoon naps. Although spindle density was lower during naps than nights, the two measures were highly correlated. For both groups, naps and nights provided highly reliable estimates of spindle density. We conclude that naps provide an accurate, reliable and more scalable alternative to measuring spindle density overnight.

KEYWORDS

measurement reliability, nap studies, polysomnoraphy, schizophrenia, sleep spindles

## 1 | INTRODUCTION

Sleep spindles, defining oscillations of stage 2 non-rapid eye movement sleep (N2), mediate memory consolidation (Fogel & Smith, 2011). Spindle activity is a stable, heritable feature of the electroencephalogram (EEG, Purcell et al., 2017), leading to its description as an electrophysiological fingerprint (De Gennaro, Ferrara, Vecchio, Curcio, & Bertini, 2005). Schizophrenia patients and their first-degree relatives have reduced spindle density (spindles/minute), which correlates with impaired sleep-dependent memory consolidation, positive symptoms, executive dysfunction and lower IQ (Manoach, Pan, Purcell, & Stickgold, 2016). Sleep spindles are the product of thalamocortical circuits and spindle density inversely correlates with thalamocortical connectivity (Baran et al., 2019). In schizophrenia, lower spindle density is associated with abnormally increased connectivity, consistent with other evidence of thalamocortical circuit dysfunction (Manoach & Stickgold, 2019). Given that spindle deficits may reflect the pathophysiology of schizophrenia and contribute to its manifestations, spindles are a promising novel treatment target for clinical trials (Wamsley et

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al., 2013). Measuring sleep spindles is also important for basic studies of memory. A major impediment to measuring spindles, however, is that overnight sleep studies are expensive, time consuming and require considerable infrastructure and participant burden. Afternoon naps may provide a more cost-effective, scalable alternative to overnight studies, if they allow reliable measurement of spindles. Following lunch there is an increase in sleep propensity, making napping feasible (Monk, 2005). Naps can enhance memory, sometimes as much as at night (Mednick, Nakayama, & Stickgold, 2003), and post-nap memory improvement correlates with spindle density (Schmidt et al., 2006). In the present study, we investigated whether naps can reliably estimate nocturnal spindle density in health and schizophrenia.

## 2 | METHODS

### 2.1 | Participants

Fourteen schizophrenia outpatients and eight healthy controls were included based on having successfully completed both overnight and napping sleep studies. Patient and control groups did not differ in age, mean parental education or estimated premorbid verbal IQ, although the control group had proportionately more females (Table 1). Patients had been maintained on stable doses of antipsychotic and adjunctive medications and one was unmedicated for at least 6 weeks prior to enrollment in both studies (see Table S1 for individual patient characteristics). Diagnoses were confirmed with structured clinical interviews for diagnostic and statistical manual of mental disorders, fourth edition (DSM-IV). Controls were screened to exclude a history of mental illness or a family history of schizophrenia spectrum disorders or psychosis. All participants were screened to exclude diagnosed sleep disorders, treatment with sleep medications, pregnancy and a history of head injury, neurological disorder or substance abuse or dependence within the past 6 months. Participants gave written informed consent and were paid for participation. The study was approved by the Partners Human Research Committee.

### 2.2 | Procedure

Participants completed four nights of polysomnography (PSG) at the Massachusetts General Hospital Clinical Research Center as part of a double-blind, randomized, placebo-controlled study of eszopiclone. Placebo and eszopiclone visits were separated by 1 week and took place on two consecutive weeknights, with the first night of each visit serving as a baseline night and the second as a memory night. On the memory night participants were trained on the finger tapping motor sequence task (MST, Wamsley et al., 2012) 2 hr before bedtime. Bedtime was 22:30 hours and participants slept for up to 10 hr. The present report includes data from the two placebo nights only.

Then, 7.4 ± 8.7 (*M* ± *SD*, range: 0.4–34.4) months later, the same participants completed three afternoon nap visits, separated by at least 1 week, with simultaneous EEG and magnetoencephalography (MEG; Elekta-Neuromag) recording of sleep. The first (adaptation) visit acclimated the participant to napping in the MEG scanner. Baseline and memory visits followed in a counterbalanced order. During the memory visit, participants trained on the MST and then had a 90-min nap opportunity starting at 14:00 hours.

### 2.3 | Polysomnography

Overnight PSG was acquired at 400 Hz using an Aura LTM64 system (Grass Technologies, Astro-Med Inc.) and a 58 channel EEG cap (Easycap GmbH, Herrsching, Germany). During naps, PSG was acquired at 600 Hz using a 70-channel Easycap. Impedances were kept below 10 kOhms at the start of each recording. Submental EMG (electromyography) and EOG (electrooculography) were also acquired.

**TABLE 1** Participant characteristics and group comparisons of demographic data

| | Schizophrenia patients (*n* = 14) | Healthy controls (*n* = 8) | | |
|---|---|---|---|---|
| | *M* ± *SD* | *M* ± *SD* | *t* | *p* |
| Age (years) | 33 ± 6 | 31 ± 6 | −0.8 | .43 |
| Sex[a] | 11M/3F | 4M/4F | NA | .34 |
| Mean parental education (years) | 14 ± 3 | 14 ± 2 | 0.1 | .92 |
| Premorbid verbal IQ[b] | 104 ± 10 | 109 ± 8 | 1.3 | .21 |
| PANSS total[c] | 63 ± 22 | NA | *Severity: mild* | |

[a]Group sex differences tested with Fisher's exact test; *M*, males, *F*, females. NA, not applicable.
[b]Estimate based on standard scores on the reading subtest of the Wide Range Achievement Test 3.
[c]PANSS (Positive and Negative Syndrome Scale) symptom severity.

Polysomnography recordings were divided into 30-s epochs and visually scored as WAKE, REM, N1, N2 or N3 according to standard criteria (Iber, Ancoli-Israel, Chesson, & Quan, 2007) by raters blind to diagnosis. Data were re-referenced to a common average and pre-processed using BrainVision Analyzer 2.0 (BrainProducts) for overnight studies and minimum norm estimate software (MNE) (Gramfort et al., 2014) and custom Matlab scripts for naps. Data were filtered at 0.3–35 Hz. Electrodes displaying significant artifacts were interpolated with spherical splines. Data were visually inspected and epochs with artifacts were removed. Five PSG records (4.6% of total) were excluded from further analyses, including three patient naps, one control nap and one control overnight, based on having either <2 min of total sleep time (TST), no N2 remaining after artifact rejection or artifacts throughout the recording.

## 2.4 | Spindle detection

Spindles were automatically detected in the 12–15-Hz band at Cz using an automated wavelet-based algorithm that was validated against hand-counted spindles in healthy and schizophrenia samples from a prior study (Wamsley et al., 2012). These samples were used to set our threshold for spindle detection to nine times the median signal amplitude of artifact-free epochs based on its maximization of between-class ('spindle' versus 'non-spindle') variance. We chose to measure N2 spindle density because it most consistently shows deficits in schizophrenia and correlates with memory (Manoach & Stickgold, 2019).

## 2.5 | Control analyses

To examine whether MST training affected spindle density or sleep architecture, we used mixed-effects linear models for nights and naps separately with subject as a random effect and visit (baseline or memory), group (controls or patients) and their interaction as fixed effects. In this model, we substituted nap order (first, second or third) for visit to examine its effects. Because there were no significant effects of MST or nap order on spindle density or any index of sleep architecture (all *p*s > .07), these factors were omitted from subsequent analyses.

## 2.6 | Nap versus night effects

We characterized differences in spindle density and sleep architecture between naps and nights using a linear mixed-effects model with subject as a random effect and group, condition (nights or naps) and their interaction as fixed effects. We also correlated average spindle density for naps and nights.

## 2.7 | Nap versus night reliability of spindle density

To calculate intraclass correlation coefficients, we estimated between- and within-subject variances in spindle density from regression models with subject as a random effect. To compare the reliability of spindle density between nights and naps we estimated the 95% confidence intervals (CIs) of $ICC_{Night}$, $ICC_{Nap}$ and their difference ($ICC_{Night}$-$ICC_{Nap}$) based on 10,000 bootstrap samples.

**TABLE 2** Nap versus night effects on sleep quality and architecture

| | Night | | Nap | | Group | Condition | Group × Condition |
|---|---|---|---|---|---|---|---|
| | Patients | Controls | Patients | Controls | | | |
| | *M* ± *SD* | *M* ± *SD* | *M* ± *SD* | *M* ± *SD* | *p* | *p* | *p* |
| Sleep quality | | | | | | | |
| TST (min) | 511 ± 47 | 500 ± 34 | 49 ± 17 | 70 ± 18 | .52 | $<10^{-3}$* | .04* |
| WASO (%)[a] | 5 ± 4 | 4 ± 5 | 37 ± 38 | 14 ± 13 | .10 | $<10^{-3}$* | .01* |
| Sleep architecture (%) | | | | | | | |
| N1 | 9 ± 5 | 10 ± 5 | 34 ± 18 | 24 ± 11 | .24 | $<10^{-3}$* | .03* |
| N2 | 58 ± 8 | 51 ± 5 | 60 ± 13 | 54 ± 11 | .11 | .47 | .91 |
| N3 | 18 ± 8 | 19 ± 6 | 5 ± 8 | 17 ± 16 | .07 | $<10^{-3}$* | .01* |
| REM | 16 ± 3 | 20 ± 5 | 1 ± 3 | 5 ± 5 | .01* | $<10^{-3}$* | .76 |
| Sleep architecture (min) | | | | | | | |
| N1 | 44 ± 23 | 47 ± 22 | 16 ± 10 | 16 ± 6 | .79 | $<10^{-3}$* | .74 |
| N2 | 296 ± 50 | 257 ± 33 | 30 ± 13 | 37 ± 13 | .12 | $<10^{-3}$* | .001* |
| N3 | 89 ± 39 | 96 ± 31 | 3 ± 4 | 13 ± 13 | .29 | $<10^{-3}$* | .75 |
| REM | 82 ± 13 | 101 ± 28 | 1 ± 2 | 4 ± 4 | .01* | $<10^{-3}$* | .01* |

Abbreviations: TST, total sleep time; WASO, wake time after sleep onset.

[a]WASO (%) was measured as WASO/(TST + WASO).

*Significant effects.

## 3 | RESULTS

### 3.1 | Nap versus night sleep quality and architecture

Patients had less TST than controls during naps (Table 2; naps: $F(1, 19) = 7.5$, $p = .01$) but not nights ($F(1, 20) = 0.3$, $p = .58$; group by condition: $F(1, 103) = 4.3$, $p = .04$). In both groups, percentage of wake time after sleep onset (WASO) was greater during naps than nights (controls: $F(1, 30) = 6.6$, $p = .02$; patients: $F(1, 54) = 55.1$, $p < 10^{-3}$; combined: $F(1, 84) = 42.9$, $p < 10^{-3}$) and more so in patients (group by condition: $F(1, 84) = 7.7$, $p = .007$).

Both groups had a higher N1% ($F(1, 84) = 77$, $p < 10^{-3}$) and minutes ($F(1, 84) = 98$, $p < 10^{-3}$) during naps than nights, and the N1% difference was greater in patients (group by condition: $F(1, 84) = 4.9$, $p = .03$). Although N2% did not differ between groups or conditions, a group by condition interaction ($F(1, 82) = 11.1$, $p = .001$) reflected that patients had more N2 minutes than controls at night ($F(1, 20) = 3.8$, $p = .07$) and fewer during naps ($F(1, 20) = 1.7$, $p = .20$). Patients ($F(1, 54) = 28.1$, $p < 10^{-3}$), but not controls ($F(1, 30) = 0.2$, $p = .66$), had a lower N3% during naps than nights (group by condition: $F(1, 84) = 7.2$, $p = .01$). Only 14/40 patient naps (35%) contained N3 compared with 18/23 control naps (78%; $\chi^2 = 10.9$, $p < 10^{-3}$). REM% was lower in naps than nights for both groups ($F(1, 84) = 297.8$, $p < 10^{-3}$) and lower for patients than controls ($F(1, 21) = 8.4$, $p = .01$). Fewer patient naps (4/40, 10%) than control naps (10/23, 44%) contained REM sleep ($\chi^2 = 9.5$, $p = .002$).

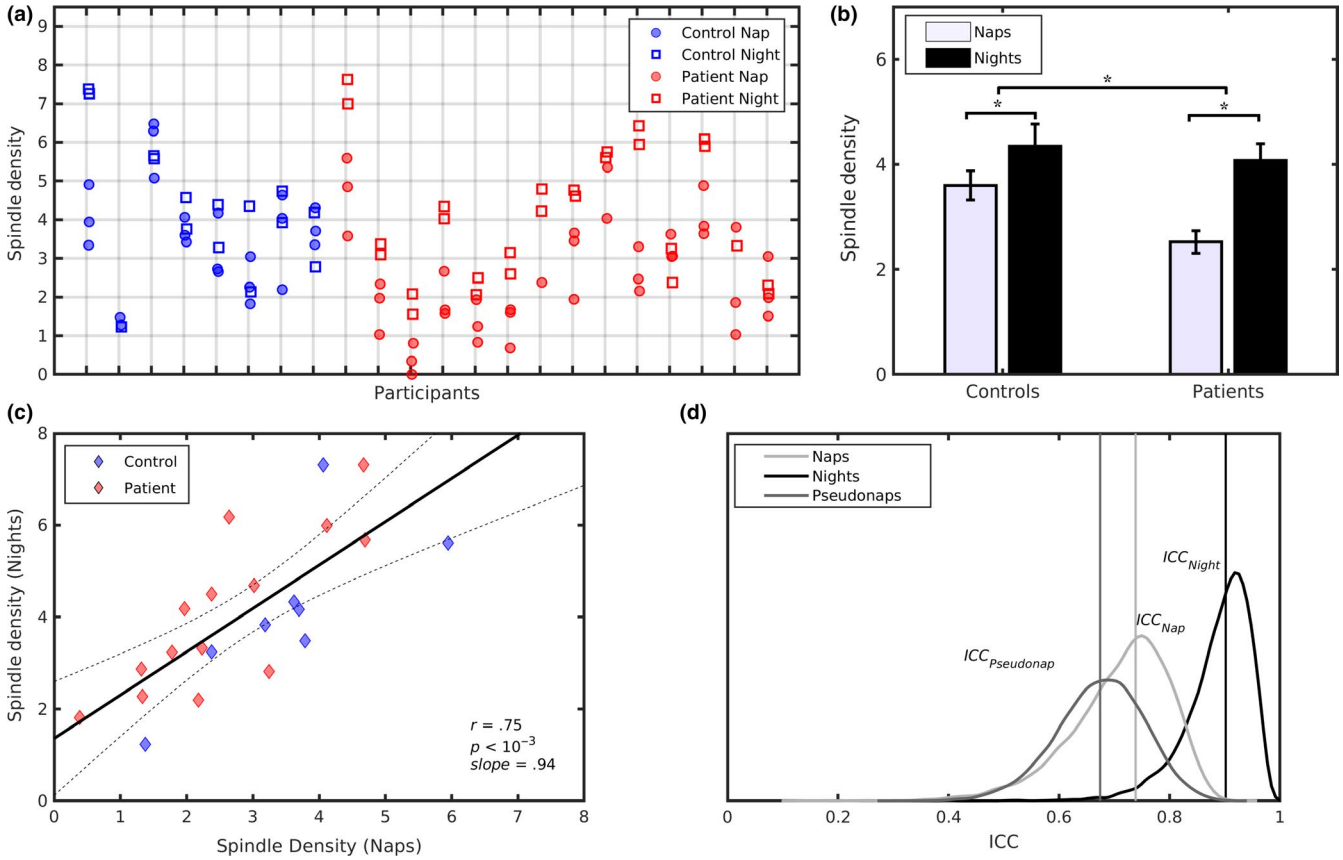


**FIGURE 1** (a) Dot plot of spindle density for each subject during naps (circles) and nights (squares). (b) Differences in spindle density between naps and nights for controls and patients. Patients showed a larger nap/night spindle density difference than controls. (c) Relation of average spindle density during naps (x-axes) and nights (y-axes). The solid line is the least squares line for the combined groups and 95% confidence intervals are indicated by dotted lines. (d) Spindle density reliability across naps ($ICC_{Nap}$), nights ($ICC_{Night}$) and pseudonaps ($ICC_{Pseudonap}$) is indicated by vertical lines along with their bootstrap distributions

**TABLE 3** Intraclass correlation coefficients (ICCs) and their 95% confidence intervals

| | Night | Nap | Pseudonap | Night–Nap | Nap–Pseudonap |
|---|---|---|---|---|---|
| Patients | 0.97* [0.92, 0.98] | 0.72* [0.39, 0.84] | 0.69* [0.46, 0.86] | 0.25* [0.11, 0.57] | 0.03 [−0.35, 0.28] |
| Controls | 0.72* [0.07,0.91] | 0.72* [0.15, 0.89] | 0.66* [0.40, 0.87] | −0.006 [−0.79,0.6] | 0.06 [−0.55, 0.37] |
| Combined | 0.89* [0.74, 0.97] | 0.74* [0.50, 0.85] | 0.67* [0.51, 0.82] | 0.17 [−0.03, 0.39] | 0.07 [−0.21, 0.27] |

*Significance ($p < .05$) based on 10,000 bootstrap samples.

### 3.2 | Nap versus night spindle density

Although spindle density was lower during naps than nights ($F(1, 81) = 40.3$, $p < 10^{-3}$) and more so for patients (group by condition: $F(1, 81) = 5.4$, $p = .02$; Figure 1a,b), average spindle density across naps and nights was highly correlated in the combined group ($r = .75$, $p < 10^{-3}$, *slope* = 0.94; Figure 1c) and for controls ($r = 0.76$, $p = .03$, *slope* = 1.02 ± 0.36) and patients ($r = .81$, $p < 10^{-3}$, *slope* = 1.08 ± 0.23) separately. The slopes did not differ between groups ($p = .89$).

### 3.3 | Nap versus night reliability

The ICCs for naps were the same across groups, but higher for patients for nights ($p = .007$; Table 3). We combined groups to increase statistical power and found that spindle density reliability was higher for nights ($ICC_{Night} = 0.89$, $p < 10^{-3}$; Figure 1d) than naps ($ICC_{Nap} = 0.74$, $p < 10^{-3}$) and this difference approached significance ($ICC_{Night}$-$ICC_{Nap} = 0.17$, $p = .09$).

We evaluated whether this difference in reliability was simply due to having less N2 during naps. For each participant, we measured spindle density in random samples of N2 from the baseline night that matched the N2 duration of each nap (pseudonap). We repeated this procedure 10,000 times to estimate the mean and 95% CI of the $ICC_{Pseudonap}$ and compared its reliability to actual naps. Spindle density reliability across pseudonaps ($ICC_{Pseudonap} = 0.67$, $p < 10^{-3}$) did not differ from actual naps in the combined group ($ICC_{Nap}$-$ICC_{Pseudonap} = 0.07$, $p = .71$; Table 3, Figure 1d).

## 4 | DISCUSSION

Measures of N2 spindle density during naps are highly reliable in both health and schizophrenia. Moreover, spindle density during naps and nights is strongly correlated, supporting the use of naps as an accurate and reliable alternative to measuring overnight spindle density, with a few caveats.

Spindle density reliability was marginally lower for naps than nights, a difference that was driven by patients. This less reliable estimate may simply reflect less N2 during naps than nights. When nocturnal N2 was sampled in nap-long segments (pseudonaps), spindle reliability for nocturnal sleep and naps did not differ.

Although N2% did not differ between nights and naps, naps significantly underestimated nocturnal spindle density. This is consistent with prior work and may reflect that melatonin secretion at night increases spindles (Knoblauch, Martens, Wirz-Justice, Krauchi, & Cajochen, 2003) or that worse sleep quality during naps impeded spindle generation (although WASO% did not correlate with spindle density). Poorer sleep quality during naps than nights in the present study may reflect having to sleep lying supine in the bore of an MEG scanner instead of a bed.

Despite having similar sleep architecture and quality during nights as controls, patients had poorer sleep quality during naps. Nevertheless, the groups did not differ in N2% or in the reliability of spindle density during naps. Although the small sample left us underpowered to examine group differences in spindle density or correlations with memory, studies with larger samples have shown that spindle density during naps is reduced in schizophrenia and correlates with memory improvement in healthy individuals (Mylonas et al., 2017; Schmidt et al., 2006).

In summary, these findings provide additional evidence that spindle density is a reliable and robust trait of the nocturnal sleep EEG (Purcell et al., 2017) and extend these findings to naps and to schizophrenia. Despite the limitations of the present study, including the long interval between overnight and nap studies and their different settings, naps still provided reliable and accurate estimates of nocturnal sleep spindle density. We conclude that naps provide a feasible, efficient and more scalable alternative to measuring spindle density in nocturnal sleep for basic and clinical studies.

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### AUTHOR CONTRIBUTIONS

All authors have made substantial contributions to the conception and design of the study, and acquisition, analysis and interpretation of data.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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