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Association of actigraphy-derived circadian phase indicators with the nadir of spindle frequency

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ABSTRACT

The measurement of chronotype and circadian rhythms in polysomnography (PSG) studies is unresolved as no validated PSG markers have been published before. Data suggest that overnight changes in sleep spindle frequency (SSF) are due to a time-of-day effect, the nadir reflecting the middle of the biological night. In this study, we tested the nadir of sleep spindle frequency (NSSF) as a phase angle estimate of the circadian rhythm. The associations between NSSF, Munich Chronotype Questionnaire (MCTQ) and actigraphy-derived sleep midpoints were analysed in a healthy young adult sample ($N = 31$; 16 females). MCTQ sleep midpoints on workdays, furthermore all actigraphy-derived sleep midpoint metrics and the least active 5 hours were consistent with the individual differences in NSSF, highlighting the potential use of NSSF as a chronotype indicator. Although further validation is needed, these results could open new horizons in the role of PSG recordings in circadian rhythm research

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EEG; sleep spindle frequency; wrist actigraphy; MCTQ; chronotype; circadian rhythm

1. Introduction

In such a highly organized and complex organism like the human body, timing has a fundamental role in maintaining proper functioning. Just like time itself, biological timekeeping systems express some kind of cyclicity. One crucially important cyclicity relies on the self-regulated circadian rhythm with a period-length of approximately 24 hours. This periodicity can be found in several biological and behavioural processes (e.g. core body temperature, melatonin production or the alternation of rest-activity periods). Given the similar period length of the circadian rhythm and of the natural light-dark cycle, as well as the fact that the main time cue (Zeitgeber) modulating circadian phase is light, the internally driven rhythm gets synchronized with the Earth's rotation around its axis. There are interindividual differences in this synchronization, that is, in the phase angle of the phase relationship between the environmental and circadian rhythm. Along these differences in the phase angle of the rest-activity period, the concept of chronotype can be discussed. Chronotype is a biological construct (Roenneberg et al. 2019), tightly related to the circadian rhythm. It refers to the entrainment of the circadian clock to the 24-hour light-

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dark cycle and reflects, among others, the individual differences in the timing of wakefulness and sleep, patterns of activity and rest over a 24-hour period, and peak performance times.

In sleep research and medicine, the measurement of the circadian rhythm would be essential as it is one of the two main sleep regulatory processes (Borbely 1982). However, the current role of polysomnography (PSG; gold-standard sleep recording method) in the evaluation of circadian rhythm disturbances is insignificant (Chokroverty et al. 2005) as there is no validated and approved PSG marker for this process yet. Non-PSG-based estimations of the phase of the circadian rhythm were indeed developed. For example, the assessment of chronotype with subjective methods (questionnaires) or with several days long rest-activity measurement. The former is accepted as a research tool, while the latter is approved by the American Academy of Sleep Medicine for the examination of circadian sleep-wake disorders (Smith et al. 2018). The Munich Chronotype Questionnaire (MCTQ) is one of the most frequently used self-assessed questionnaires characterized by the evident advantage of asking about bed- and wake times (on both free and workdays) instead of preferences, which might be answered more objectively (Roenneberg et al. 2003). Regarding actigraphy, it can be said that it is an objective tool for examining the rest-activity rhythm. However, a person's activity during a 24-hour day can be easily "contaminated" by non-regular individual choices, such as studying at night before an exam or going out with friends, etc. These social and work-related activities are masking the person's circadian rhythm and, therefore, may bias its measurement. Thus, for an appropriate estimate, it requires more than 2–3 days long recordings.

Although not directly validated yet, a recent paper by Bódizs et al. (2022) raised the possibility that the frequency of the sleep spindles (one of the main characteristics of non-rapid eye movement sleep electroencephalography (NREM EEG) signal) shows circadian rhythmicity. This hypothesis was based on earlier findings suggesting the circadian modulation of sleep spindle frequency (SSF). For example, the nadir of SSF (NSSF) was shown to overlap with core body temperature minimum (Wei et al. 1999), just as with the acrophase of salivary melatonin rhythm (Knoblauch et al. 2005). Furthermore, a study found faster spindles during daytime naps compared to spindles recorded during night sleep periods (Rosinvil et al. 2015), suggesting a time-of-day effect in SSF. One of the main goals of our former study was to show that the circadian process can be revealed from just one baseline night polysomnography recording with the examination of the overnight dynamics of SSF. The results were in correspondence with earlier findings. We found higher daytime nap SSFs relative to night time SSFs, the age-related changes in circadian modulation of SSFs were proven, in addition, an overnight U-shaped behaviour was revealed (lower SSF in the middle, compared to the beginning and the end of the night). Finally, a new metric was introduced, namely the NSSF. NSSF was defined as a measure that reflects the estimated time when SSF reaches its minimum during the sleep period. As the above findings imply that NSSF might also be considered as a phase angle estimate of the circadian rhythm, the aim of the present study is to test this metric against actigraphy and MCTQ, which are already accepted chronotype indicators. Our hypotheses are the following:

(1) SSF in sleep cycle (NREM period) 2 and 3 will be lower than in cycles 1 and 5/6: NSSF will be in the middle of the sleep period
(2) NSSF will correlate positively with the MCTQ- and actigraphy-derived sleep midpoints: e.g. subjects with earlier chronotype indicators according to the MCTQ and actigraphy measures will have earlier NSSF as well
(3) Sample means of the different time variables (MCTQ, actigraphy sleep midpoints, and NSSF) will not differ significantly, as all these variables reflect the middle of the sleep period.

2. Methods

2.1. Sample and study protocol

This study was part of a larger research project focusing, among others, on the effects of sleep deprivation in $N = 40$ healthy young adults. Subjects in the age range of 18–40 years of age were enrolled by a combination of convenience and snowball sampling procedures involving personal contacts and social media calls. All subjects were free of psychiatric or neurological disorders based on self-reports. In addition, the exclusion criteria of the study included the Hungarian version of Pittsburg Sleep Quality Index (Takács et al. 2016) score over 5, Beck Depression Inventory (Beck et al. 1961) score over 12 (moderate and severe depression symptoms (Rózsa et al. 2001), alarm clock usage on free days, extreme circadian preference (MCTQ chronotype scores outside of the ± 2 SD of reported values in young Hungarian subjects according to Haraszti et al. (2014) and shift work, as well as reported acute and/or chronic medical diagnoses or ongoing pharmacological treatments. On average, 37.45 days (SD: 36.4 days) after the completion of the questionnaires, eligible participants were enrolled in a 7-day long protocol involving actigraphy, headband-recorded mobile home PSG, and sleep deprivation. Here, the pre-sleep deprivation phase of the study was analysed: the first 6 calendar days of actigraphy (5 nights) and the baseline night of headband-PSG data, as well as the MCTQ data. An additional exclusion of 9 participants was due to poor quality baseline EEG recordings or complete data loss. Hence, the final sample consisted of $N = 31$ subjects (age range: 18–39 years, 16 females).

Recordings took place between April and December in Hungary. Participants had to wear an accelerometer for 7 days on their non-dominant wrist. No instructions were given for the first 4 days except not to take off the actigraphy device for any activity. Thus, these measurements give an insight into the real rest-activity rhythm of the participants. On the 5th day, participants had to come to the laboratory to learn how to use the mobile PSG device. On this same evening (5th night of the experiment), they had to put on the headband for themselves at home with the help of online guidance. The bedtime of baseline sleep was freely chosen by the participants; thus, they could go to bed according to their own preferences. The use of an alarm clock was prohibited in the mornings of headband-PSG-recorded sleep.

National Public Health Centre Institutional Committee of Science and Research Ethics approved the research protocols, and the experiment was implemented in accordance with the Declaration of Helsinki. Every participant signed an informed consent about their attendance in the study.

2.2. Munich chronotype questionnaire

MCTQ measures usual bed- and wake times on working and free days and the middle of the sleep period is calculated separately for the two variables (MSW, MSF as mid sleep on work/free days, respectively). Finally, the oversleeping adjusted sleep midpoint on free days is considered as the chronotype indicator (MSFsc) (Roenneberg et al. 2003).

2.3. Electroencephalography (EEG)

Sleep EEG was measured with the Zmax EEG headband (Hypnodyne Corp., Sofia, Bulgaria), including derivations F7-Fpz and F8-Fpz which were re-referenced to their common average. The sampling frequency was 256 Hz. Other relevant technical specifications are the 16-bit sampling precision and 0.1–128 Hz bandwidth. After scoring the EEG records in 20-second epochs, a 4-second based visually guided manual artefact removal was performed. Determination of sleep cycles was based on the modified Feinberg & Floyd criteria (Feinberg and Floyd 1979) proposed by Jenni and Carskadon (2004) with the consideration of skipped REMs in the first cycle of sleep. Artifact-free NREM sleep periods of successive sleep cycles from the baseline night were included in the analyses.

2.3.1. Determination of sleep spindle frequency (SSF) with the individual adjustment method of sleep spindle detection (IAM)

In coherence with our earlier study (Bódizs et al. 2022), SSF was determined by the IAM-approach, which consists of several steps. The whole procedure can be seen in the paper of Bódizs et al. (2009). Here the main steps are described. First, the average NREM sleep EEG amplitude spectra are computed in the 9–16 Hz range by using a Fast Fourier Transformation routine. Then, the individual adjusted frequency limits of slow and fast sleep spindles are determined based on the second-order derivatives of the amplitude spectra. Here, the two spectra of the two signals are the same due to the common average reference. Frequency limits will be the zero crossing points of the negative peaks. Finally, the middle-frequency is used as spindle frequency which is the arithmetic mean of the individual-specific lower and upper frequency limits. This procedure was applied in every cycle separately to get the individual cycle-specific spindle frequency middles for each participant. In this report only slow spindle frequencies were obtained and analyzed as there is a well-known region-specificity of slow and fast spindles and only anterior EEG channels were available.

2.3.2. Phase of the nadir of sleep spindle frequency: NSSF

Phases of the nadirs of SSFs (NSSF) were estimated as follows. First, the lowest SSF-containing sleep cycle was identified for each participant separately then, the local time of the middle of this respective sleep cycle was determined (Bódizs et al. 2022).

2.4. Actigraphy

Activity was measured with Activinsights Geneactiv Original wristband accelerometer. The device measured acceleration from x-, y-, and z-axes with 100 Hz sampling frequency (except one participant for whom the sampling rate was 75 Hz). The R package GGIR (Van

Hees et al. 2015; Migueles et al. 2019) was applied to analyse the accelerometer data with different approaches such as quantifying some of the well-known nonparametric measures of the rest-activity rhythm (see below), and defining the sleep period time window (Van Hees et al. 2015). The time window over which summary statistics are derived was set to WW (wake to wake); furthermore, the measurement after the baseline EEG night was excluded from the analyses to avoid biases in activity data due to the sleep deprivation protocol. With these settings 29 participants resulted in 5 and 2 participants in 6 analysable days and nights. The sleep midpoint analyses were performed on the first 5 sleep period windows of 29 participants, whereas the 2nd to the 6th night was used in the case of 2 subjects (because their recordings were started a day before than the others). That is, the baseline EEG night was the last analysed sleep period by actigraphy. The nonparametric estimates were computed on 4 consecutive full WW periods for 29 participants and on 5 for 2 subjects, ending with the awakening from the baseline night.

2.4.1. Nonparametric measures

2.4.1.1. Interdaily stability (IS), Intradaily variability (IV). IS quantifies the invariability between the days, whereas IV gives an indication of the fragmentation of the rhythm. Both metrics are calculated with GGIR which follows the original approach by Van Someren et al. (1999), but is slightly modified in the case of IV (GGIR considers the epoch transitions between the end of a day and the beginning of the next day in a different way, as compared to the original approach).

IS gives an indication of the synchronization to the 24-h light-dark cycle. That is the measure reflects the strength of the coupling of the rest-activity rhythm to environmental zeitgebers. IS is around 0 for Gaussian noise, and 1 for perfect stability.

IV indexes the fragmentation of the circadian rhythm, that is higher IV indicates more napping during daytime and more awakenings during the nights. IV values are near 0 for perfect sine waves and about 2 for Gaussian noises.

2.4.1.2. Least and most active 5 hours. The start time and the average acceleration were calculated for the least and the most active 5 hours for the first 5 days independently. Then, 8 variables were derived: the average of the least and most active 5 hours' mean (L5_avr, M5_avr, respectively), the mean start time of the periods (L5time_avr, M5time_avr) and the 5th day metrics (L5_5th, M5_5th, L5time_5th, M5time_5th). The 5th day was treated as a separate variable in the analyses as this is the closest in time to the NSSF and thus, may reflect it better.

2.4.2. Determination of the sleep period time window and sleep midpoint variables

The determination of the sleep period time window from acceleration data was based on the heuristic algorithm of Van Hees et al. (2015). This algorithm first calculates the arm angle from the 3 acceleration sensors (x, y, z) in rolling 5-second time window, then averaging it in 5-second epochs. After that, the change in arm angle is calculated from epoch to epoch. Finally, if the change was no larger than 5° for 5 minutes, the period was considered as a potential sleep period. After the sleep period estimation, we used the sleep onset and wake-up times (start and end time of sleep period time window) to estimate the actigraphy version of the MCTQ variables described in subheading 2.2. The fifth experimental night (i.e. baseline

EEG night) was considered a free day as participants were instructed to switch off their alarm clocks and wake up spontaneously. Hence, the 3 actigraphy-derived sleep midpoint variables were the following: average sleep midpoints of the first 4 nights as work-day sleep midpoint (ActMSW), the 5th night's midpoint as free day sleep midpoint (ActMSF), and the oversleeping adjusted sleep midpoint (ActMSFsc).

2.5. Statistical analysis

Prior to any calculations or statistical testing, all time variables were transformed to fractional numbers as follows: 1 hour = 1/24. The values after midnight (and before noon) were increased by 1 to handle midnight crossovers.

All statistical analyses were carried out with Statistica 13 software. Associations of different variables were tested by using the Pearson product-moment correlation analyses on normally distributed or logarithmically transformed (log-normalized) variables. We also tested if there is a systematic difference between the parallel measures of the circadian phase by using dependent sample t-tests on normally distributed data and Wilcoxon Matched Pairs Test on variables with non-Gaussian distribution. The test of normality was performed by the Shapiro-Wilk test for all variables.

The threshold of significance was defined as $p < 0.05$ in all analyses.

3. Results

All variables were normally distributed except NSSF ($W = 0.93$, $p = 0.04$), IS ($W = 0.92$, $p = 0.02$), M5 value in the 5th day ($W = 0.73$, $p < 0.001$), EEG awakening time ($W = 0.93$, $p = 0.04$), EEG falling asleep time ($W = 0.92$, $p = 0.03$), actigraphy falling asleep time ($W = 0.93$, $p = 0.04$) and spindle frequency in cycle 5 ($W = 0.83$, $p = 0.001$) according to Shapiro-Wilk test. After natural-based log-transformation, all variables became normally distributed.

3.1. Sleep cycles and NSSF

All subjects ($N = 31$) had at least 4 (mean: 5.29), $N = 12$ had 5, $N = 11$ had 6, and 2 participants had 7 complete sleep cycles. The sample mean of lnNSSF was 0.13 ($SD = 0.066$; $\exp(M_{lnNSSF}) = 1.139 \sim$ **03:20 hh:mm**). The mean sleep cycle in which the sleep spindle frequency reached its minimum was 2.65. From all participants, 3 subjects had their NSSF in the first, 10 in the second, 14 in the third, 3 in the fourth and 1 in the fifth sleep cycle. Furthermore, we analysed if there were significant differences between the "middle" and the first/last sleep cycles in terms of spindle frequency. We considered cycles 2–3 the middle, and 5–6 the end of the night as the mean number of sleep cycle was 5.29 in the sample. SSF in cycle 2 ($m = 11.93$, IQR = 11.53, 12.53) and 3 ($m = 11.99$ Hz, IQR = 11.61, 12.36) was significantly lower than in cycle 5 ($m = 12.18$, IQR = 11.98, 12.53; $T_{c2c5} = 30$, $Z_{c2c5} = 3.6$, $p < 0.001$; $T_{c3c5} = 27$, $p < 0.001$) and 6 ($m = 12.26$, IQR = 12.02, 12.82; $T_{c2c6} = 5$, $Z_{c2c6} = 2.8$, $p = 0.005$; $T_{c3c6} = 3$, $p = 0.003$). First cycle SSF ($m = 12.1$, IQR = 11.67, 12.44) did not differ significantly from SSF in cycle 2 ($T_{c1c2} = 158$, $Z_{c1c2} = 1.8$, $p = 0.08$), but a significant SSF decrease was observed from cycle 1 to 3 ($T_{c1c3} = 141$, $Z_{c1c3} = 2.1$, $p = 0.036$) (see Figure 1).

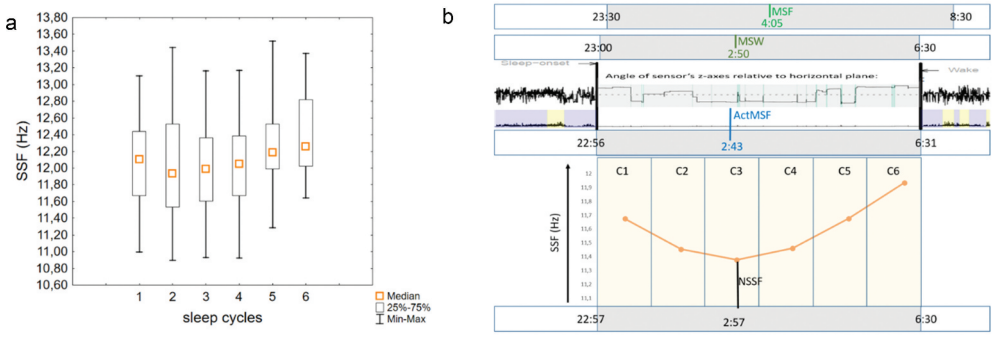

Figure 1. *Median of SSF in different sleep cycles and an example for sleep midpoint variables, the timing of NSSF and the overnight dynamics of SSF in a 26-year-old male participant*. A. Overnight dynamics of SSF follow a U-shaped dynamic (orange rectangles indicate the medians of SSF in the different sleep cycles) B. The grey areas within the four blue rectangles indicate the sleep windows of the participant according to the different measures. From the bottom to top: 1. Is the sleep window according to the EEG on a free day, 2. sleep period based on actigraphy estimates (note the high correspondence with EEG), 3. self-reported sleep window on work days, 4. self-reported sleep period on free days. The orange line represents the cycle dynamics of slow spindle frequencies in the different sleep cycles (C1-C6). Finally, the time-points in different colours index the timing of different sleep midpoint variables and NSSF in this particular individual.

3.2. Sleep period window: EEG vs. Actigraphy

The average sleep duration according to EEG-based scoring was $M = 7{:}58{:}47$ (range: 05:53–10:43 hh:mm); however, the actigraphy-estimated sample mean sleep period window on the same night (5th actigraphy-measured night) was slightly longer: $M = 8{:}24{:}30$ (range: 5:52–12:25 hh:mm). Co-registered EEG and actigraphy on night 5 revealed significant earlier estimated sleep onset times for actigraphy ($T = 58$, $Z = 3.7$, $p < 0.001$), but no significant difference in the timing of final awakenings ($T = 150$, $Z = 1.9$, $p = 0.055$). The mean differences in the sample were $M = 0{:}19{:}21$ hh:mm:ss (min: 00:00:00, max: 2:48:55), and $M = 0{:}20{:}08$ (min: 00:00:20, max: 3:23:10) regarding awakening and falling asleep, respectively. A difference of more than 10 minutes was present in only 9 subjects with respect to wake-up times and in 10 subjects in the falling asleep metrics.

The difference between the sleep duration of work- and free days was tested. Neither the EEG-derived ($t = -0.9$, $p = 0.4$, $M_{EEG} = 7{:}58$ hhmm, SD = 01:10 hhmm), nor the actigraphy-measured ($t = 0.8$, $p = 0.4$, $M_{Actfree} = 8{:}24$ hhmm, SD = 01:26 hhmm) free day sleep durations were significantly different from the actigraphy-estimated average workday sleep period length (mean of the 1–4th night; $M = 8{:}11$ hh:mm, SD = 00:57 hh:mm).

3.3. Interdaily stability, intradaily variability

The sample average was $M_{lnIS} = -0.44$ (SD = 0.17, $\exp(M_{lnIS}) = 0.64$), and $M_{IV} = 0.58$ (SD = 0.17). No sex differences were revealed (lnIS: t = 1.32, p = 0.2, $M_f = -0.41$, $M_m = -0.49$; IV: $t = -1.92$, p = 0.07, $M_f = 0.52$, $M_m = 0.65$).

3.4. Consistency between the time variables

The time of the NSSF was significantly positively correlated with actigraphy-estimated sleep midpoints, MCTQ MSW, as well as with the onset of the least active 5 hours (both with L5time_avr and L5time_5th). Thus, the subjects with an earlier self-assessed workday-, actigraphy estimated sleep midpoints, and least active 5 hours had their NSSF earlier as well (Figure 2). The beginning of the most active 5 hours in the 5th day correlated negatively, while MCTQ MSF, MCTQ MSFsc and M5time_avr did not show significant correlation with NSSF (Table 1).

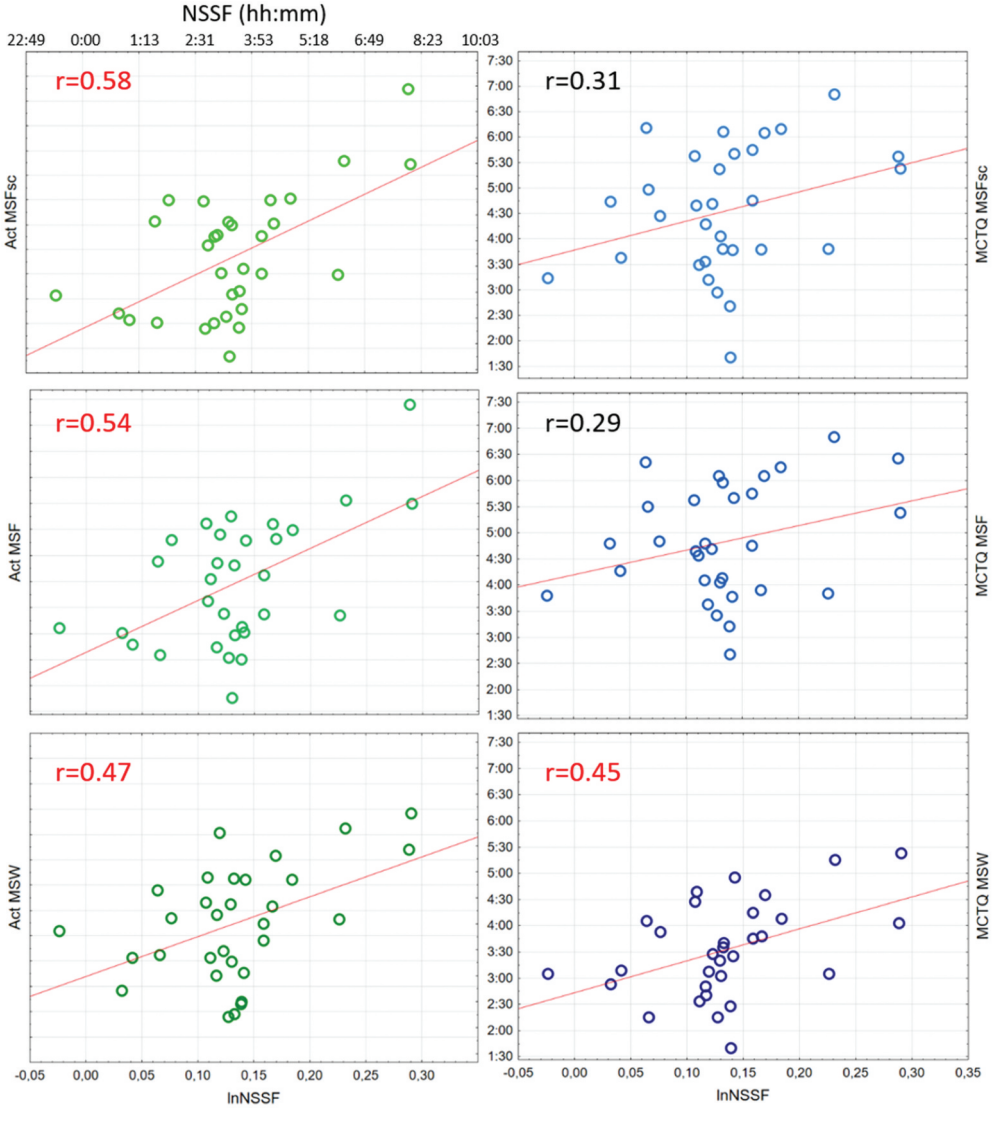

Figure 2. *Scatterplots and Pearson correlation coefficients (r) depicting the relationship between different sleep midpoint variables and NSSF*. red coefficient values indicate significant correlations ($p < 0.05$).

Table 1. Person's r and respective p-values between NSSF and sleep midpoint metrics.

		MCTQ MSW	MCTQ MSF	MCTQ MSFsc	Act MSW	Act MSF	Act MSFsc	L5 time average	L5 time 5th day	M5 time average	M5 time 5th day
NSSF	r	**0.45**	0.29	0.31	**0.47**	**0.54**	**0.58**	**0.52**	**0.42**	−0.16	**−0.40**
	p	**0.012**	0.117	0.092	**0.008**	**0.002**	**0.001**	**0.002**	**0.019**	0.394	**0.027**

Table 2. Medians and interquartile ranges of different time variables.

	median	lower quartile	upper quartile
Act MSW	4:09:10	3:20:33	4:53:52
Act MSF	4:02:30	3:00:37	4:54:00
Act MSFsc	3:27:53	2:35:32	4:23:07
MCTQ MSW	3:27:30	2:52:30	4:07:30
MCTQ MSF	4:45:00	3:54:00	5:45:00
MCTQ MSFsc	4:39:00	3:37:25	5:37:30
NSSF	3:20:30	2:45:10	4:08:00

To further analyse the unexpected association regarding M5 time variables to NSSF, we checked their relation to the other (subjective and objective) circadian phase estimators. Neither the actigraphy variables (ActMSF, ActMSW, ActMSFsc), nor the MCTQ indicators correlated significantly with M5time_avr and M5time_5th.

Act MSW, Act MSF, Act MSFsc significantly correlated with MCTQ MSW ($r = 0.7$, $p < 0.001$), MCTQ MSF ($r = 0.62$, $p < 0.001$), MCTQ MSFsc ($r = 0.5$ $p = 0.005$), respectively.

3.5. Is there a difference between the different sleep-midpoint indicators and NSSF?

Times of the NSSF differed significantly from the chronotype indicator of MCTQ (MSFsc, $T = 106$, $Z = 2.8$, $p = 0.005$) and the midpoint of sleep on free days (MSF, $T = 76$, $Z = 3.4$, $p < 0.001$). However, NSSF seemed to fall in the middle of the night if the self-assessed sleep midpoint on workdays (MSW, $T = 247$, $Z = 0.02$, $p = 0.984$) and actigraphy-estimated midpoint metrics (ActMSW, $T = 153$, $Z = 1.9$, $p = 0.06$, ActMSF, $T = 161$, $Z = 1.7$, $p = 0.09$, ActMSFsc, $T = 216$, $Z = 0.6$, $p = 0.53$) were considered, as it was not significantly different from these measures. Act MSW, Act MSF, Act MSFsc were significantly different from MCTQ MSW ($T = 56$, $Z = 3.8$, $p < 0.001$), MCTQ MSF ($T = 65$, $Z = 3.6$, $p < 0.001$), and MCTQ MSFsc ($T = 90$, $Z = 3.1$, $p = 0.002$), respectively. See medians and interquartile ranges in Table 2.

4. Discussion

The aim of the current study was to test the appropriateness of the NSSF in reflecting the estimates of already accepted chronotype metrics. Overall, we found that sleep midpoint indicators derived from actigraphy are consistent with the time of the sleep spindle frequency minima. Sample medians were similar, and actigraphy variables reliably reflected the individual differences in NSSF. In turn, among subjective indicators, only sleep midpoints on workdays revealed the same relationships with our new EEG measure proposed herein.

4.1. The timing of sleep spindle frequency minimum

The deceleration of sleep spindles in the middle of the sleep period was demonstrated in studies with different chronobiological protocols, manipulations of sleep timing, or with large datasets (Aeschbach et al. 1997; Wei et al. 1999; Bódizs et al. 2022). In the present study we could replicate these findings, as 90% of our sample expressed a minimum SSF in the 2nd or 3rd sleep cycle (sample mean of total sleep cycle numbers was 5.29). In addition, significant differences between the SSFs of the middle vs. first/last sleep cycles were revealed (as we assumed in hypothesis 1.). Most of the former studies revealing a U-shaped distribution of SSF during night sleep linked this behaviour of SSF to circadian modulation or a time of day effect. However, except our own earlier study (Bódizs et al. 2022) none of the reports attempted to use the nadir of this U-shape as a phase angle estimate of the circadian rhythm. Thus, our aim was to test whether this behaviour coheres with the phase angle of the actigraphy-derived or questionnaire-based estimation of the circadian rhythm. Testing was performed on a healthy sample with normal circadian rhythms as reflected by the relatively large synchronization (IS) and small fragmentation (IV) metrics as well as by standard MCTQ MSFsc values according to formerly reported statistics (Haraszti et al. 2014).

4.2. Correlation of the different sleep midpoint metrics and NSSF

The assumption of the positive correlation between NSSF and other circadian phase estimators was almost completely fulfilled (hypothesis 2). NSSF could reveal the inter-individual differences in MCTQ MSW, actigraphy time variables and in the onset of least active 5 hours. That is, people with earlier SSF minimum tend to have their MCTQ MSW, actigraphy-derived sleep midpoints, and least active 5-hour periods earlier as well. However, this was not the case regarding other MCTQ midpoints and the most active 5 hours.

The fact that the MCTQ MSF and MSFsc were not in line with objectively measured midpoints of sleep might be interpreted in several ways. Although the test-retest reliability of this questionnaire has already been proven in different studies (Reis et al. 2020; Wang et al. 2023), one influential factor might be that MCTQ was one of the screening questionnaires in the original study, thus participants filled it out earlier than the experiment began. However, it is interesting that MCTQ MSW was characterized by the strongest correlation with actigraphy sleep midpoints, uncovering similar medians as Act MSFsc and NSSF. Furthermore, MCTQ MSW is the only subjective variable which was correlated with NSSF. One possible explanation is that the schedule of young adults' free days is so variable that they can describe their workdays by specifying usual bedtimes with higher precision than their free day bedtimes. A similar interpretation was provided in an earlier study with an emphasis on the intraindividual variability of sleep midpoints which revealed stronger correlations between average daily self-assessed workday midpoint and MCTQ MSW than between daily self-assessed free day sleep midpoints and MCTQ MSF (Lenneis et al. 2021). The difference between correlations regarding work and free days was stronger when actigraphy and daily self-assessed sleep midpoints were tested. The authors explained these results as participants can remember their sleep times better on workdays than in free days. However, Santisteban et al. (2018) investigated the relationship between

MCTQ and actigraphy, and found reliable correlation between free day-, but not between workday midpoints. Findings were assumed to reflect the tendency of the participants to report idealized sleep patterns for workdays instead of the real ones (Santisteban et al. 2018). Thus, it cannot be ruled out that objective metrics in the present study reflect only workdays as the definition of free day was somehow arbitrary (prohibited alarm clock use). However, the first explanation seems a little more plausible as MCTQ also puts a great emphasis on alarm clock usage: if someone reports using an alarm clock on free days, their chronotype cannot be calculated (Roenneberg et al. 2012). All in all, this question needs further investigation where the date of completing the questionnaire is better controlled.

Regarding the most active 5 hours, our results were a little confusing. Indeed, in the literature instead of the 5, the most active 10 hours is usually used. We chose the shorter period in order to provide a more accurate estimate of the timing of accumulated maximal activity during a day. As we could not find explanation for those results, we tested whether the already accepted circadian phase estimators are associated with it. Our assumption was that the timing of the maximal activity during the day reflects diurnal time preference, namely the time-of-day when the participants are the most alert (morningness-eveningness (Horne and Ostberg 1976)), which is tightly related to the construct of chronotype (Zavada et al. 2005). A recent study suggests that older people with later chronotypes time their main activities later in the day (Hicks et al. 2023). However, in the present study the association of this narrower activity period with NSSF contradicts our expectations. Nevertheless, the timing of the most active 5 hours was not revealed to be a reliable estimate of time preference or chronotype, as it did not correlate with any of the chronotype indicator in the present study. One possibility is that the activity of younger participants is more tainted by social and work-related factors, so they do not have the chance to time their maximal activity according to their preferences.

4.3. Sleep period window and sleep midpoints according to the different measures

The assumption of similar sample means of different time variables (hypothesis 3) was based on the idea that all those metrics reflect the middle of the habitual sleep periods. Findings revealed a similar pattern to the results obtained by the correlational analyses. Consequently, we have successfully demonstrated comparable medians for actigraphy-derived sleep midpoint metrics and NSSF, and the subjectively assessed midpoints were significantly different from the EEG-based measure. Likewise, the comparison of actigraphy-derived and MCTQ-based sleep midpoints was dissimilar, which contradicts earlier studies revealing comparable means for these variables (Jankowski 2016; Santisteban et al. 2018; Wang et al. 2023). Our result suggests that the subjectively estimated bed and wake times differed from that of the objectively measured ones in the present dataset. Indeed, EEG- and actigraphy-derived bed- and wake times revealed convergent results. Although the actigraphy- and EEG-based estimations of the timings of sleep onset were statistically different, two-thirds of the subjects had lower than 10-minute difference between these measures. As polysomnography is the gold-standard method of sleep detection, we can conclude that the definition of sleep period window from actigraphy data was approximately reliable. The dissimilarity of subjective and objective sleep timings in our sample suggests that the issue of their convergence needs further investigations in larger datasets and/or more controlled environments.

4.4. Strengths and limitations

The main strength of this study is that it provides a new perspective in the field of sleep EEG research. Our findings suggest that besides the homeostatic regulation, sleep EEG might contain information on the circadian phase as well. However, no direct proof for the association of our candidate EEG-measure of circadian phase with gold-standard phase markers (like DLMO or core body temperature minimum) is available at the current stage. Another limitation is the retrospective nature of the study. It was not designed for the assessment of chronotype directly, thus the date of the questionnaire completion, the season of data collection, and the instructions for sleep timing during the week of objective measurements were not strictly defined. Finally, the so-called first night effect (well-known from polysomnography literature (Agnew et al. 1966)) could influence our findings, although participants could sleep in their usual environment (in their own bed), which cancels one of the main factors of this phenomenon. Furthermore, sleep duration of the headband recording night was not lower when compared to the other days' sleep amount.

Despite the above-mentioned limitations, it can be concluded that the time of the sleep spindle frequency minimum is convergent with the actigraphy-derived objective estimators of the circadian phase, a result which could open new horizons in the role of sleep EEG recordings in circadian rhythm research.

Disclosure statement

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Author contribution

C. G.H. designed and conceptualized the study, performed analyses, collected the data, interpreted the results and wrote the paper. R. B. designed and conceptualized the study, interpreted the results and wrote the paper. Both authors revised and approved the final version and agree to be accountable for all aspects of the work.

Data availability statement

The dataset analysed during the current study is available here: OSF repository.

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