

Age-Related Changes in the Circadian Modulation of Sleep-Spindle Frequency During Nap Sleep

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Study Objectives: Sleep spindles exhibit a clear circadian modulation in healthy younger people. During the biological night (when melatonin is secreted), spindle density and spindle amplitude are high and spindle frequency and its variability are low, as compared with the biological day. We investigated whether this circadian modulation of spindle characteristics changes with age.

Design: A 40-hour multiple-nap paradigm under constant-routine conditions

Setting: Chronobiology Laboratory, University Psychiatric Hospitals, Basel, Switzerland

Participants: Seventeen younger (20-31 years) and 15 older (57-74 years) volunteers.

Interventions: N/A.

Measurements and Results: Whereas the circadian modulation of spindle density, amplitude, duration, and intraspindle frequency variability was not greatly affected by age, we found significant changes in the circadian modulation of spindle frequency. The pronounced circadian modulation of

spindle frequency in younger, but not older, subjects was phase locked with the circadian rhythm in melatonin secretion. In the latter, circadian modulation was attenuated and tended to be advanced with respect to the timing of melatonin secretion. There was no difference between age groups in the phase of the sleep-wake cycle or that of melatonin, nor did the phase angle between them differ. Although changes in the circadian modulation of spindle frequency in older subjects were accompanied by reduced amplitude in the sleep consolidation profile, there was no significant correlation between spindle frequency and sleep consolidation.

Conclusion: This multiple-nap protocol under constant-routine conditions revealed an age-dependent weaker coupling of the circadian rhythms of spindle frequency and sleep propensity to the circadian rhythm of melatonin secretion.

Keywords: Sleep spindles, EEG, aging, melatonin, circadian rhythms

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INTRODUCTION

SLEEP SPINDLES ARE TRANSIENT 12- TO 15-HZ OSCILLATIONS, WHICH, TOGETHER WITH SLOW WAVES, HALLMARK THE HUMAN NON RAPID EYE MOVEMENT (NREM) sleep electroencephalogram (EEG). They originate in the thalamus and are generated in a thalamocortical network as a result of membrane hyperpolarization.¹ In contrast to slow waves, sleep spindles are subject to a pronounced circadian modulation. During the biological night, spindle density and spindle amplitude are high, while spindle frequency is low.^{2,3} EEG power density in the spindle frequency range exhibits a shift in peak frequencies across the circadian cycle. Highest power density in lower-frequency activity (12.25-13 Hz) is found during melatonin secretion and shifts to a higher frequency range (14.25-15.5 Hz) when melatonin is not secreted.⁴ Neither the mechanism by which the circadian signal influences sleep spindles nor its significance is yet known. It has been suggested that sleep spindles may gate synaptic transmission through the thalamus to the cortex and

thereby display a sleep protective function.¹ Indeed, there is a tight temporal association between the phase of melatonin secretion—during which sleep spindles of high amplitude and low frequency are abundant—and the level of sleep consolidation (as indexed by percentage of wakefulness during sleep episodes).⁴ It has therefore been hypothesized that the circadian modulation of sleep spindles could be a mechanism by which the circadian pacemaker enhances sleep consolidation during the biological night.⁴

Sleep consolidation is attenuated with age, as indexed by reduced sleep efficiency,⁵⁻⁷ more awakenings,^{8,9} and an increase in the percentage of light sleep (stage 1) at the expense of deep sleep (slow-wave sleep).^{5,7} It is not yet clear whether age-related changes in sleep are due to alterations in the homeostatic and/or the circadian regulation of sleep and wakefulness, or their interactions. Reduced amplitude and advanced phase of circadian rhythms with age have been reported, for example, in core body temperature,^{7,10,11} melatonin,¹² cortisol,¹³ and sleep propensity.^{6,14} However, the literature is controversial, and the use of different methodologies to investigate the contributions of the 2 processes (normal entrained conditions, constant-routine, or forced-desynchrony protocols) and subject selection may have contributed to this inconsistency (see references 15 and 16 for reviews).

Sleep spindles are affected by the aging process. Reduced spindle density, amplitude, and duration and, generally (but not always), a slight increase in spindle frequency^{2,17-20} as well as a reduction of spindle frequency activity (spectral EEG power density) below 14 Hz⁵ have been reported. This raises the question whether these changes in sleep spindles are related to reduced sleep consolidation in aging. In addition, the circadian regulation of sleep spindles is affected by age. In a forced-desynchrony study, spindle density, frequency, amplitude, and duration exhib-

Disclosure Statement

This was not an industry supported study. Drs. Knoblauch, Münch, Blatter, Martens, Schröder, Schnitzler, Wirz-Justice, and Cajochen have indicated no financial conflicts of interest.

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ited a clear circadian variation in younger subjects, while, in an older age group, only spindle frequency exhibited a circadian modulation.²

In the present study, we analyzed sleep spindles in naps distributed over the circadian cycle to test the hypothesis that the circadian modulation of spindle characteristics is attenuated with age. Furthermore, we aimed at assessing the relationship between age-related changes in sleep spindles and sleep consolidation.

METHODS

Study participants

Seventeen healthy younger (9 women, 8 men, age range 20–31 years, mean: 25 ± 0.9 SEM) and 15 healthy older (7 women, 8 men, age range 57–74 years, mean: 65.1 ± 1.4) volunteers participated in the study. All subjects were nonsmokers and free from medical, psychiatric, and sleep disorders—as assessed by screening questionnaires, a physical examination, and a polysomnographically recorded screening night. For the older subjects, a neuropsychological assessment (CANTAB® test battery and the Stroop Test) was carried out to exclude motor, attention, or memory impairments. The criteria for the absence of sleep disorders were a sleep efficiency of at least 80%, fewer than 10 periodic leg movements per hour, and an apnea/hypopnoea index lower than 10. Drug-free status was verified via urinary toxicologic analysis. A morning-evening type questionnaire²¹ was used to exclude extreme chronotypes (score >23 or <12). The morning-evening type scores were slightly but significantly higher (earlier chronotype) in the older than in the younger group (mean \pm SEM: 18.8 ± 0.8 vs 16.4 ± 0.8 ; *t* test: $P < .05$). The older volunteers received a subcutaneous injection of low-dose heparin (Fragmin® 0.2 mL, 2500 IE/UI, Pharmacia AG, Dübendorf, Switzerland) on the 3 consecutive study days under constant posture conditions to prevent venous thrombosis. Younger female subjects were studied during the follicular phase of the menstrual cycle; 5 of them used oral contraceptives. All participants gave signed informed consent, and the study protocol, screening questionnaires, and consent form were approved by the local Ethics Committee and conformed with the Declaration of Helsinki.

Protocol

The protocol comprised 3.5 days in the sleep laboratory under controlled conditions: an adaptation night (BL1), a baseline night (BL2), a 40-h short sleep-wake cycle paradigm (“nap protocol”) and an 8-h recovery sleep episode (Figure 1). Continuous polysomnographic recording started in the afternoon after the adaptation night. During the 40-hour short sleep-wake cycle paradigm, subjects completed 10 alternating cycles of 75 minutes of scheduled sleep (light levels: 0 lux) and 150 minutes of scheduled wakefulness. The wake episodes were spent under constant-routine conditions (constant dim light levels <8 lux, constant posture, food, and liquid intake at regular intervals, no time cues; for details of the constant-routine method, see reference 22). During the week preceding the study (home baseline week), subjects were instructed to refrain from excessive physical activity, caffeine and alcohol consumption and to maintain a regular sleep-wake schedule (bedtimes and wake times within ± 30 minutes of self-selected target time). The latter was veri-

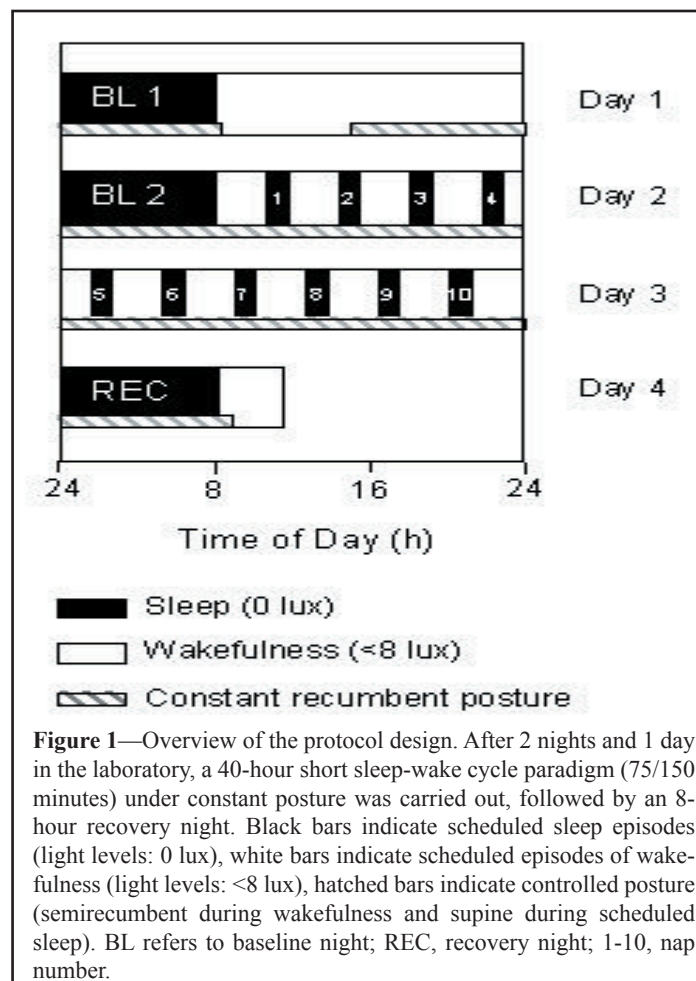


Figure 1—Overview of the protocol design. After 2 nights and 1 day in the laboratory, a 40-hour short sleep-wake cycle paradigm (75/150 minutes) under constant posture was carried out, followed by an 8-hour recovery night. Black bars indicate scheduled sleep episodes (light levels: 0 lux), white bars indicate scheduled episodes of wakefulness (light levels: <8 lux), hatched bars indicate controlled posture (semirecumbent during wakefulness and supine during scheduled sleep). BL refers to baseline night; REC, recovery night; 1–10, nap number.

fied by a wrist activity monitor (Cambridge Neurotechnologies®, UK) and sleep logs. The timing of the sleep-wake schedule in the study protocol was calculated such that the 8-hour sleep episodes were centered at the midpoint of each volunteer’s habitual sleep episode as assessed by actigraphy during the baseline week. Results from the baseline and recovery night of the younger participants have been reported elsewhere.²³

Sleep Recordings and Analysis

Sleep was recorded polysomnographically using the VITA-PORT digital ambulatory sleep recorder (Vitaport-3 digital recorder, TEMEC Instruments B.V., Kerkrade, The Netherlands). Twelve EEGs, 2 electrooculograms, 1 submental electromyogram, and 1 electrocardiogram signal were recorded. All signals were filtered at 30 Hz (fourth-order Bessel-type anti-aliasing low-pass filter, total 24 dB/Oct), and a time constant of 1.0 seconds was used prior to online digitization (range 610 μ V, 12 bit AD converter, 0.15 μ V/bit; sampling rate at 128 Hz for the EEG). The raw signals were stored online on a Flash RAM Card (Viking, Rancho Santa Margarita, Calif) and downloaded offline to a PC hard drive. Sleep stages were visually scored on a 20-second basis (Vitaport Paperless Sleep Scoring Software) according to standard criteria.²⁴ Here, we report EEG data during stage 2 sleep derived from the midline (Fz, Cz, Pz, Oz) referenced against linked mastoids (A1, A2).

EEG Instantaneous Spectral Analysis

EEGs were subjected to instantaneous spectral analysis using

the fast time frequency transform. For the EEG, the fast time frequency transform calculates instantaneous amplitude, frequency, and bandwidth in 8 frequency bands from 0-4 Hz, 4-8 Hz, 28-32 Hz with a time resolution of 0.125 seconds. Over a moving template of 1-second duration, thresholds are applied to amplitude, frequency, and bandwidth parameters to detect and differentiate synchronized activity from ongoing noise, as well as to remove artifacts. Spindles were detected from the outcome of the 8- to 12-Hz and 12- to 16-Hz frequency band, but the frequency and bandwidth threshold for spindle detection were limited to the range of 11 to 16 Hz. Furthermore, a duration limit (≥ 0.5 seconds and ≤ 2 seconds) was applied for detected spindles. As a result, the amplitude and frequency of each individual spindle is computed at a time resolution of 0.125 seconds and a 0.25-Hz frequency resolution. Furthermore, the number of sleep spindles per 20-second epoch was calculated, and, for each individual spindle, the following variables were computed: duration, mean frequency, mean amplitude, standard deviation of frequency, and frequency at onset and offset. (For further details of the method see reference 3.)

Salivary Melatonin

Saliva was collected at approximately 30-minute intervals during scheduled wakefulness. Saliva samples were assayed for melatonin using a direct double-antibody radioimmunoassay validated by gas chromatography mass spectroscopy with an analytical least-detectable dose of 0.15 pg/mL and a functional least-detectable dose of 0.65 pg/mL (Bühlmann Laboratories, Schönenbuch, Switzerland).²⁵

Classification of Naps

Naps comprising a total duration of stage 2 sleep of less than 5 minutes were excluded from the analysis. The first 75 minutes after lights off in the recovery night were considered as an additional nap. For statistical time-course analysis of the sleep-spindle variables, nap 4 and 10 (during the “wake maintenance zone”) were excluded because too few younger subjects fulfilled the criterion of a duration of stage 2 sleep of at least 5 minutes (5 younger subjects for nap 4 and 3 younger subjects for nap 10). A total of 9 subjects per age group fulfilled these criteria in the remaining naps, and data from these subjects were entered in the statistical analysis.

Naps were classified into night naps and day naps, depending on their occurrence during or outside the melatonin secretory phase. This was defined as follows: the 24-hour mean melatonin concentration (between hours 5 and 29 of the 40-hour nap protocol) was calculated for each subject as an individual threshold level. A nap was rated as a night nap if the melatonin concentration of the last saliva sample immediately (within 5 minutes) before the nap was above the threshold; otherwise, it was rated as a day nap. There were, on average, 5.8 ± 0.4 day naps and 2.9 ± 0.2 night naps per subject in the younger and 7.1 ± 0.3 day naps and 2.9 ± 0.2 night naps in the older age group. For both age groups, the duration of stage 2 sleep did not significantly differ between day and night naps ($P > .1$, 1-way analysis of variance [ANOVA]), and there was no significant interaction between age and condition ($F_{1,30} = 0.59$; $P = .45$; 2-way ANOVA). (For more details on sleep stages across the naps see reference 26.)

Statistics

The statistical packages SAS® (SAS Institute, Inc., Cary, NC; Version 6.12) and Statistica® (StatSoft Inc. 2000. STATISTICA for Windows, Tulsa, Okla) were used. For day-night comparisons, averaged values across daytime naps were compared with averaged values across nighttime naps. One-, 2- and 3-way ANOVAs were used. All *P* values derived from ANOVAs were based on Huynh-Feldt corrected degrees of freedom, but the original degrees of freedom are reported.

Cross-correlation analysis between the melatonin and spindle-frequency rhythms were performed for each individual. First, melatonin concentration values in the 3.75 hours around the time point of the spindle frequency value were averaged, and then the spindle frequency curve was displaced by 3.75-hour steps (lags) relative to the fixed melatonin curve. Cross correlations were calculated for each time lag. A linear trend subtraction procedure [$f(x) = x - (a + b) \cdot t$, where *a* and *b* were automatically estimated from the data, STATISTICA time series module] and a moving average procedure over 3 data points was applied to the spindle-frequency values before entering the cross-correlation analysis, although a cross-correlation analysis on untrended values yielded very similar results (data not shown). All individual correlation coefficients were Fisher *z* transformed before averaging over subjects, and the resulting mean correlation coefficients were retransformed. Correlation coefficients were considered significant if they exceeded ± 2 SEM.

The time course of spindle frequency was fitted with a sinusoidal function comprising the fundamental oscillation (24-hour component): $f(t) = y_0 + A \cdot \sin(2 \cdot \pi \cdot t / \tau_{24} + c)$. In this model, 4 parameters were estimated: *A* represents the amplitude, τ_{24} the period, *c* the phase position, and y_0 the intercept of the fitted sine curve. Data were fit with a nonlinear least-square fitting analysis based upon the Marquardt-Levenberg algorithm to find the coefficients (parameters) of the independent variable or variables that give the best fit between the equation and the data (SigmaPlot for Windows, Version 7.0, Richmond, Calif; Systat Software, Inc). The goodness of fit of each sine fit was assessed by calculating the adjusted correlation coefficient (R^2) and the power, or the probability that the model correctly describes the relationship of the variables, if there is a relationship. Sine fits with power values < 0.65 were classified as not reliable sine fits. This threshold was set after visual inspection of each single fit by 2 of the authors (VK and CC). The adjusted correlation coefficients (R^2) for the reliable sine fits ranged between 0.30 and 0.95.

RESULTS

Sleep Timing and Melatonin Secretion

Sleep Timing

The timing of the sleep-wake schedule during the protocol was calculated for each participant based on his or her sleep times during an ambulatory baseline week preceding the study (see Methods). In the study protocol, the bed times and wake-up times of older subjects were, on average, 23 minutes earlier than those of the younger subjects (mean timing of scheduled sleep episode \pm SEM: 11:11 PM - 7:11 AM \pm 10 minutes vs 11:34 PM - 7:34 AM \pm 14 minutes; NS, $P = .19$; Mann-Whitney U test).

Table 1—Three-way Analysis of Variance With the Factors Age, Condition, and Derivation for Spindle Density, Frequency, Amplitude, Duration, and Intraspindle Frequency Variability

	Density		Frequency		Amplitude		Duration		ISFV	
	F	P	F	P	F	P	F	P	F	P
A	23.74	*	3.06	†	21.51	*	27.67	*	39.35	*
C	44.76	*	17.32	*	14.39	*	0.08	.78	37.19	*
D	52.41	*	185.89	*	84.73	*	35.15	*	63.65	*
AxC	5.7	*	17.13	*	1.03	.32	0.02	.89	2.72	.11
AxD	13.1	*	2.56	†	11.75	*	7.21	*	1.75	.18
CxD	42.9	*	2.72	†	4.17	*	2.06	.11	4.39	*
AxCxD	5.01	*	0.81	.47	1.89	.14	3.96	*	0.52	.62

A refers to age; C, condition (day, night); D, derivation (Fz, Cz, Pz, Oz); ISFV, intraspindle frequency variability.

* $P < .05$, † $P < .1$

Melatonin

Older subjects had significantly lower melatonin secretion (average 24-hour value) than the young subjects (5.2 ± 0.7 vs 9.1 ± 1.4 pg/mL; $P < .05$; 2-tailed t test for independent samples). The midpoint of melatonin secretion occurred at $2:37$ AM ± 17 minutes in the older and $3:02$ AM ± 12 minutes in the younger subjects (NS, $P = .58$, Mann-Whitney U test).

Phase Relationship Between Sleep Timing and Melatonin Midpoint

The time interval between melatonin midpoint and scheduled wakeup time did not differ significantly between the older and younger age groups (4.56 ± 0.25 hours vs 4.53 ± 0.22 hours; $P = .98$ Mann-Whitney U test).

In summary, the circadian phase of the sleep-wake cycle, melatonin midpoint, and the phase angle between the sleep-wake cycle and melatonin secretion did not differ statistically between age groups.

Sleep Spindles

Day-Night Difference

In a first step, day-night differences in spindle variables were assessed. After classification of day and night naps (see Methods), spindle characteristics were first calculated per nap and then averaged over the naps within each condition (day and night). A 3-way ANOVA with the factors age (younger, older), condition (day, night), and derivation (frontal, central, parietal, occipital) was performed for the spindle variables density, frequency, amplitude, duration, and intraspindle frequency variation (Table 1). There was a significant main effect of age for all variables except for spindle frequency. Older subjects had lower spindle density and amplitude, shorter spindle duration, and higher intraspindle frequency variation compared with the younger subjects (Figure 2). The interaction between age and condition was significant for spindle density and frequency; the 3-way interaction between age, condition, and derivation was significant for spindle density and duration (Table 1).

Spindle Density

In both age groups, spindle density during the night was higher than during the day in all derivations except in Fz (posthoc com-

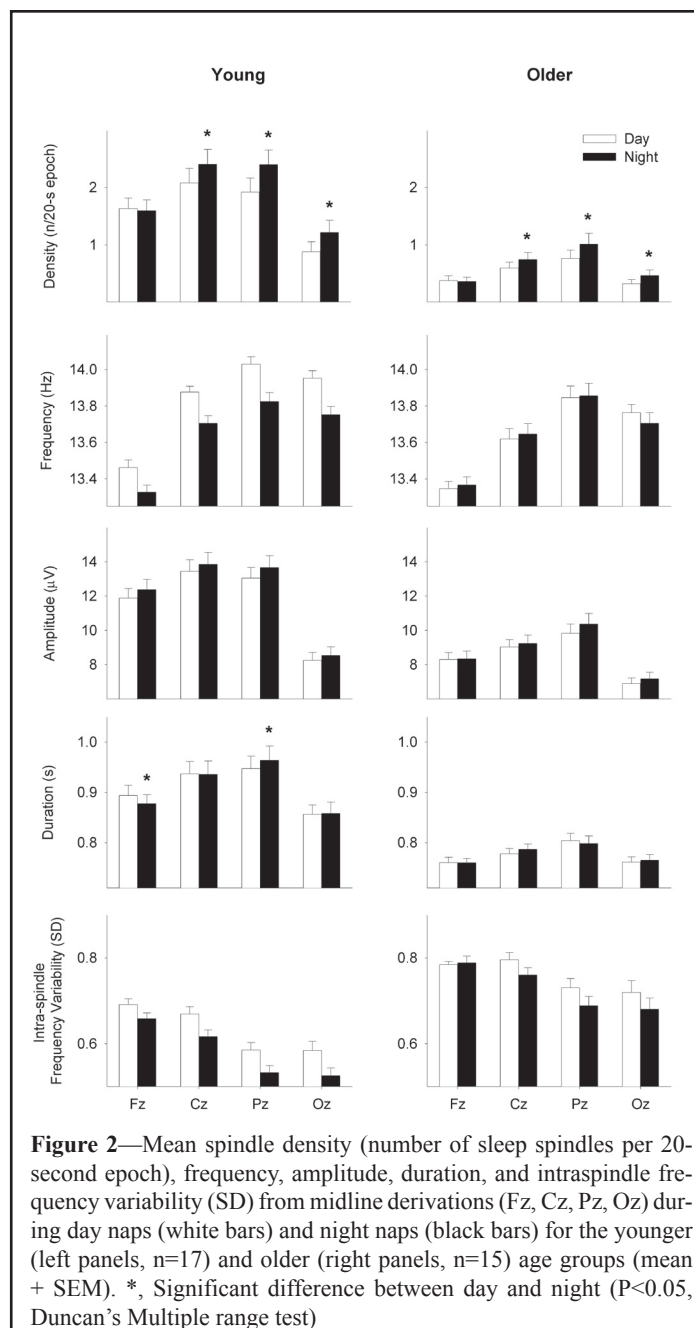


Figure 2—Mean spindle density (number of sleep spindles per 20-second epoch), frequency, amplitude, duration, and intraspindle frequency variability (SD) from midline derivations (Fz, Cz, Pz, Oz) during day naps (white bars) and night naps (black bars) for the younger (left panels, $n=17$) and older (right panels, $n=15$) age groups (mean + SEM). *, Significant difference between day and night ($P < .05$, Duncan's Multiple range test)

parisons, Duncan multiple range test). Since the significant interaction between age and condition indicated that the extent of this nocturnal increase was unequal for the age groups, the difference between spindle density during night and day naps was calculated. When pooled over all derivations, the day-night difference was significantly larger in younger than in older subjects (1-way ANOVA and Mann-Whitney U test).

Spindle Frequency

In the older subjects, spindle frequency (pooled over all derivations) did not differ significantly between day and night (Duncan multiple range test). In contrast, in the younger subjects, spindle frequency during the night was significantly lower than during the day. Both age groups had similar spindle frequencies during the night, whereas spindle frequency during the day was significantly higher in younger than in older subjects (Duncan's multiple range test).

Table 2—Three-Way Analysis of Variance With the Factors Age, Nap, and Derivation for Spindle Density, Frequency, Amplitude, Duration and Intraspinde Frequency Variability

	Density		Frequency		Amplitude		Duration		ISFV	
	F	P	F	P	F	P	F	P	F	P
A	12.4	*	1.24	0.28	15.77	*	11.47	*	11.47	*
($F_{1,16}$)										
N	4.65	*	5.23	*	5.04	*	1.93	†	6.49	*
($F_{8,128}$)										
D	31.88	*	108.54	*	48.27	*	22.63	*	25.93	*
($F_{3,48}$)										
AxN	0.49	.84	3.48	*	1.62	.13	0.75	0.64	0.72	.65
($F_{8,128}$)										
AxD	12.43	*	2.14	.13	15.34	*	5.44	*	1.72	.2
($F_{3,48}$)										
NxD	4.45	*	0.61	.87	0.88	.6	1.13	.32	1.1	.35
($F_{24,384}$)										
AxNxD	1.23	.29	1.1	.36	0.9	.59	0.97	.5	0.7	.81
($F_{24,384}$)										

A refers to age; N, nap (nap 1-11, without nap 4 and 10); D, derivation; ISFV, intraspindle frequency variability * $P<.05$, † $P<.1$

Duration

In younger subjects, spindle duration during the night was shorter than during the day in Fz and longer in Pz (Duncan multiple range test). No day-night differences in spindle duration were observed in the older group.

Time Course

The time course of spindle variables across consecutive naps is shown in Figure 3 for Cz. A 3-way ANOVA between the factors age, nap, and derivation was performed (Table 2). There was a significant main effect of age for all variables and a significant main effect of nap for all variables except for duration, for which the factor nap showed a tendency ($P<.1$). The interaction between age and nap was significant for spindle frequency. For the other variables, this interaction was not significant, indicating that the time course of these variables was not significantly affected by age. The 3-way interaction between age, nap, and derivation was not significant for any of the spindle variables. Therefore, only data from the central derivation Cz are reported below. (For regional differences in the circadian variation of these spindle variables in the younger age group, see reference 3.)

Figure 4 depicts the time course of spindle frequency together with the mean curve of melatonin secretion. In younger subjects, spindle frequency exhibited a pronounced and statistically significant variation over time (1-way ANOVA *time*, $P<.0001$). In the older subjects, the variation over time in spindle frequency failed to reach significance (1-way ANOVA *time*, $P=.05$). Visual inspection indicates that the relationship to melatonin secretion is changed in the elderly. In the younger subjects, spindle frequency is high outside the phase of melatonin secretion, declines at the onset of melatonin secretion, and increases in the morning after the offset of melatonin secretion. In the older subjects, spindle frequency begins to decrease earlier, already before melatonin is secreted, and increases earlier, when melatonin is still being secreted.

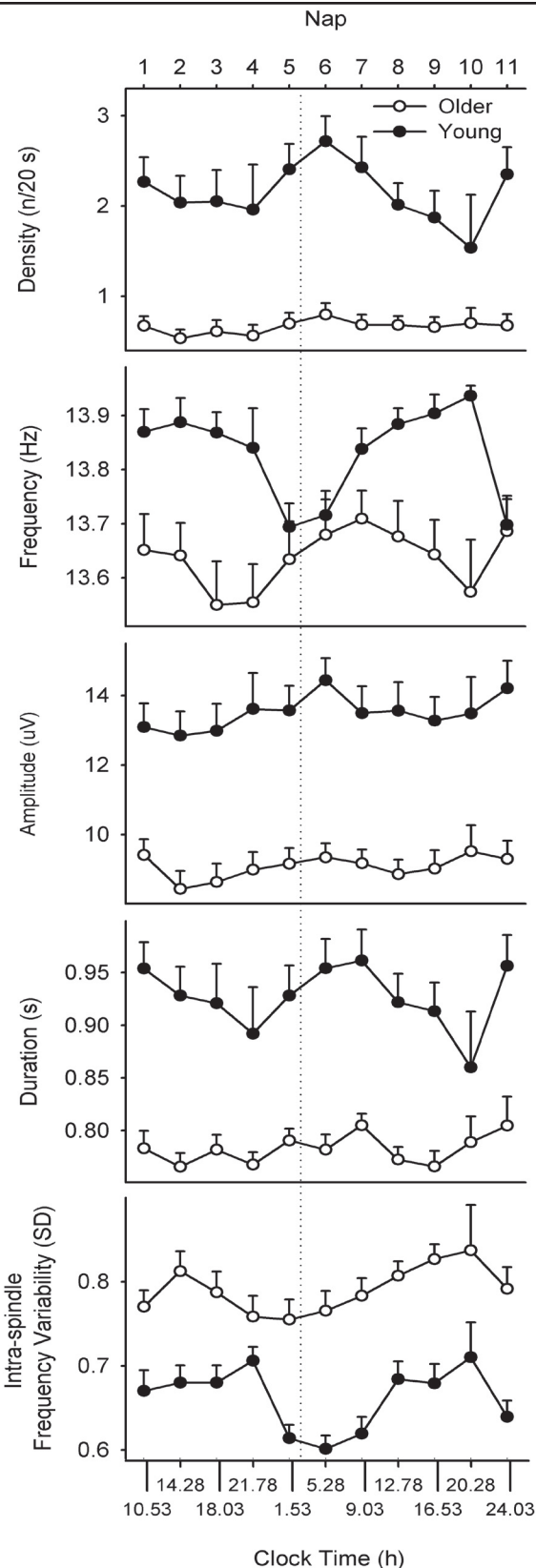


Figure 3—Time course of spindle density, frequency, amplitude, duration and intraspindle frequency variability across the naps in Cz for the younger (filled circles) and the older (open circles) age groups (mean + SEM). The vertical dotted line indicates the mean midpoint of melatonin secretion. Note that naps with less than 5 minutes of stage 2 sleep were excluded. This resulted in different numbers of subjects entering the mean for the naps. For the older age group, 10 or more subjects participated in all naps, and, in the younger group, 12 or more participated except for nap 4 ($n=5$) and nap 10 ($n=3$).

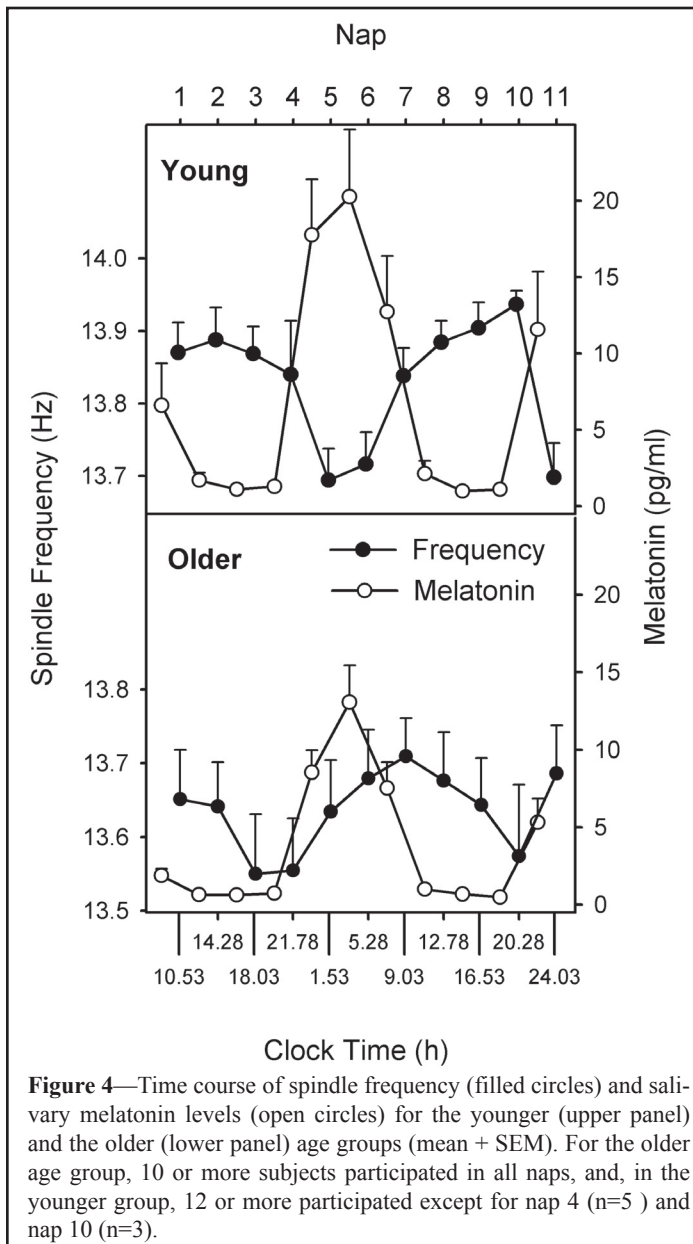


Figure 4—Time course of spindle frequency (filled circles) and salivary melatonin levels (open circles) for the younger (upper panel) and the older (lower panel) age groups (mean + SEM). For the older age group, 10 or more subjects participated in all naps, and, in the younger group, 12 or more participated except for nap 4 ($n=5$) and nap 10 ($n=3$).

Cross Correlations

To quantify the phase relationship between melatonin secretion and the time course of spindle frequency, a cross-correlation analysis between the 2 rhythms over 3.75-hour time lags was performed for each individual. Figure 5 depicts the mean cross-correlation coefficients as a function of the time lags. For the younger age group, there was a significant negative correlation between the rhythm of melatonin secretion and spindle frequency at time lags around 0 hours (between -2 and +2 hours), which indicates that the minimum of the spindle-frequency rhythm coincided with the peak of the melatonin curve. In the older age group, the shape of the curve suggests that the spindle-frequency rhythm is advanced relative to the melatonin rhythm (indicated by a nadir at positive time lag around 6-7 hours). However, correlation coefficients did not reach significance at any time lag.

Sine Fits

To estimate the phase of the frequency minimum and the am-

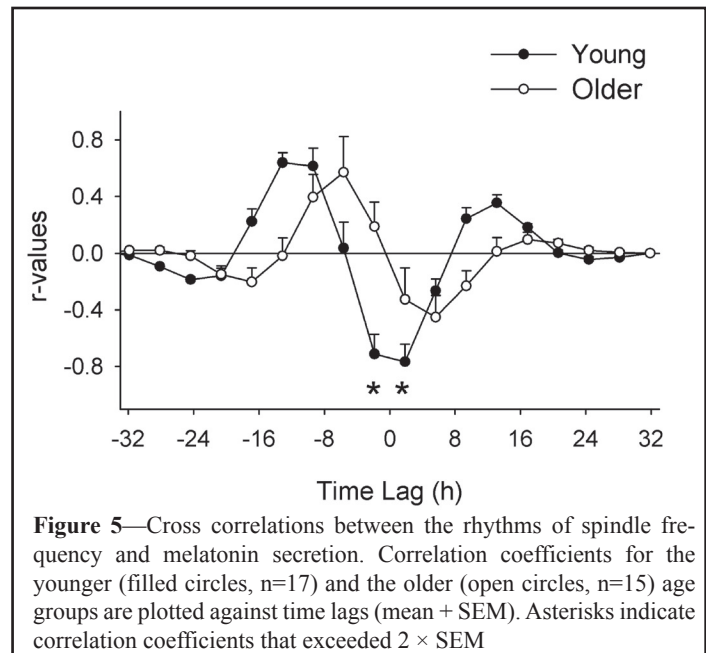


Figure 5—Cross correlations between the rhythms of spindle frequency and melatonin secretion. Correlation coefficients for the younger (filled circles, $n=17$) and the older (open circles, $n=15$) age groups are plotted against time lags (mean + SEM). Asterisks indicate correlation coefficients that exceeded $2 \times \text{SEM}$.

plitude of the spindle frequency rhythm, sine fitting was applied to the spindle-frequency curves (see Methods). In the younger age group, this was possible for 15 out of 17 subjects, whereas in the older age group, only 8 out of 15 subjects met the criteria for reliable sine fits. In the younger subjects, the phase of the fitted frequency minimum occurred on average $0.4 \text{ hours} \pm 0.5$ before the midpoint of melatonin secretion. This time interval was not statistically different from 0 ($P>.05$; t test). In the elderly, the minimum of the fitted frequency rhythm was significantly advanced by $6.8 \text{ hours} \pm 0.3$ relative to the midpoint of melatonin secretion ($P=.001$; t test), and occurred 7.19 hours earlier than in the younger group ($P=.0005$, Mann-Whitney U test). The amplitude and period of the fitted frequency rhythm did not differ between the age groups ($P=.9$ and $.7$; Mann-Whitney U test).

Relationship Between Spindle Frequency and Sleep Consolidation

Time Course of Wake Time

The time course of sleep stages across the naps is reported in detail elsewhere.²⁶ Here, only the time course of wake time (wakefulness + movement time) is reported as an index of sleep consolidation across the naps. depicts wake time per 15-minute interval after lights off during the scheduled sleep episodes. A 3-way ANOVA with the factors age, nap, and 15-minute interval was performed. There was a significant main effect of nap ($F_{10,300}=28.1$, $P<.0001$) and a significant main effect of 15-minute interval ($F_{4,120}=113.6$, $P<.0001$) but no significant main effect of age ($F=1$, $P=.3$). There was, however, a significant 2-way interaction between age and nap ($F_{10,300}=3.6$; $P=.002$) and between nap and 15-minute interval ($F_{40,1200}=4.1$, $P<.0001$) and a significant 3-way interaction between age, nap, and 15-minute interval ($F_{40,1200}=2.1$, $P=.002$). Posthoc comparisons revealed that older subjects had significantly less wake time during the second part (the last 45 minutes) of nap 4 and 10. On the other hand, they had more wake time during the second part of nap 7 (the last 30 minutes) and nap 8 (the last 45 minutes) and during the third 15-minute interval of nap 9 ($P<.05$; Mann-Whitney U test).

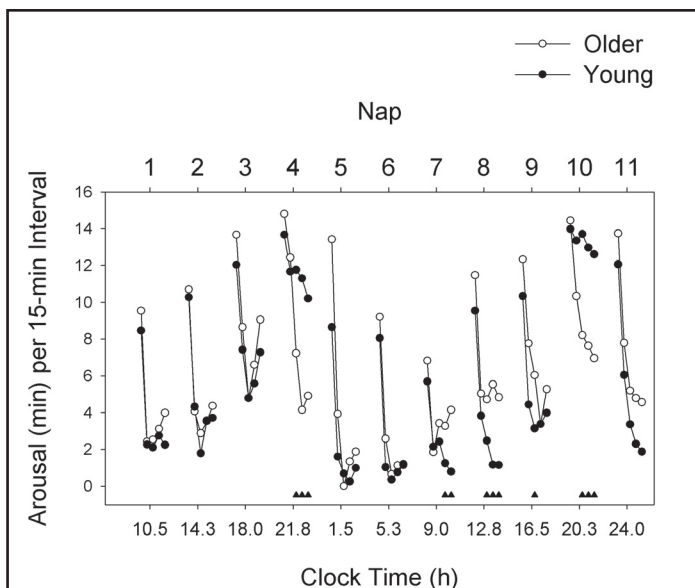


Figure 6—Time course of wake time (wakefulness + movement time) per 15-minute intervals after lights off during the scheduled 75-minute sleep episodes for the younger (filled circles, mean, $n=17$) and the older (open circles, mean, $n=15$) age groups. Triangles near the abscissa indicate a significant difference between the age groups in these 15-minutes intervals ($P<.05$, Mann-Whitney U test).

Correlation Between Spindle Frequency and Wake Time

To assess if there is a relationship between spindle frequency and the level of sleep consolidation across the naps, correlations between spindle frequency and wake time over the 11 naps was calculated for each subject separately. The mean correlation coefficient was 0.24 (range: -0.72 to 0.82) in the younger and -0.13 (range: -0.72 to 0.68) in the older age group. The same correlation analysis was performed with spindle density. Mean correlation coefficients were -0.11 (range: -0.95 to 0.65) in the younger and 0.01 (range: -0.7 to 0.85) in the older age group. Within each group, there was no significant correlation between the day-night difference in spindle frequency and the day-night difference in wake time.

DISCUSSION

Younger subjects exhibited a pronounced circadian modulation of spindle frequency, which was phase locked with the circadian rhythm of melatonin secretion. Spindle frequency was low during the biological night, when melatonin is secreted, and high outside melatonin secretion. In the older age group, the circadian rhythm of spindle frequency was less clearly pronounced, as indicated by a smaller number of subjects for whom spindle-frequency data met the criteria for reliable sine fitting. Furthermore, the spindle-frequency rhythm was no longer coupled to the circadian rhythm of melatonin secretion, as indicated by the absence of significant cross correlations between the 2 rhythms. In those older subjects for whom sine fits were applicable, the spindle-frequency minimum was markedly advanced relative to the younger age group and relative to the peak in melatonin secretion. The timing of melatonin secretion itself, as well as the phase relationship between melatonin secretion and habitual sleep times at home, did not differ between the 2 age groups.

Sleep spindles originate in the thalamus, which is the relay station for most sensory signals to the cerebral cortex (for a review

see reference 1). Their generation depends on the level of membrane hyperpolarization in thalamic neurons. At the transition from wakefulness to sleep, the membrane potential of neurons in the thalamus and cortex is progressively reduced, which allows the appearance of the characteristic NREM sleep oscillations, sleep spindles and slow waves. This hyperpolarization is associated with impaired synaptic responsiveness.^{27,28} Thereby, the flow of sensory information to the cortex is generally reduced during NREM sleep.

Besides this tonic hyperpolarization, sleep spindles may represent an additional element for reducing sensory responsiveness. Their generating mechanism involves inhibitory GABAergic projections from thalamic reticular neurons to thalamocortical relay neurons, where they elicit rhythmic inhibitory postsynaptic potentials.¹ This phasic inhibition of thalamocortical relay neurons may additionally block sensory transmission to the cortex, making sleep spindles a putative mechanism to protect the sleeping brain from arousing stimuli and thereby enhance sleep consolidation.²⁹

The frequency of sleep spindles is determined by the duration of the phasic hyperpolarization sequence elicited by the rhythmic inhibitory postsynaptic potentials in thalamocortical relay neurons. In cats, a hyperpolarization of about 70 milliseconds in duration results in spindle frequencies of approximately 14 to 15 Hz, whereas a longer hyperpolarization leads to lower frequencies.³²

There is evidence that low spindle frequencies are associated with conditions of particularly high sleep consolidation. Sleep deprivation, an intervention that enhances the homeostatic pressure for sleep, results in a reduction of spindle frequency³³ and, in the EEG power spectrum, in a decrease in power density in the high spindle frequency range^{23,33-35} and an increase in the low-frequency range.^{23,33} The circadian modulation of sleep spindles is such that low-frequency spindles are promoted during the phase of melatonin secretion, ie, during the normal time for sleep: spindle frequency is reduced,^{2,3} and EEG power density⁴ and spindle amplitude³ in the low spindle frequency range is increased as compared to the daytime. Therefore, it could be hypothesized that the circadian modulation of spindle frequency is related to a circadian signal that modulates the level of arousal and sleep propensity to facilitate a consolidated sleep bout during the night and a consolidated wake bout during the day. In the present study, the age-related changes in the circadian modulation of spindle frequency came along with a reduced amplitude in the circadian profile of sleep propensity, as indexed by the time course of wake time (Figure 4), total sleep time (stage 1-4 plus rapid eye movement sleep), sleep latency to stage 2, and subjective sleepiness (assessed by the Karolinska Sleepiness Scale).²⁶ Differences between the younger and older subjects were particularly pronounced in the evening, during the so-called 'wake-maintenance zone', when the circadian drive for sleep is particularly low.^{6,36,37} This circadian "waking signal" in the evening seems to be attenuated in the elderly, who had significantly more total sleep time, less wake time, shorter sleep latencies, and higher subjective sleepiness ratings during this time (see also reference 26). These results are in accordance with a previous study reporting an increase in sleepiness in older compared with younger subjects between 7:00 PM and 9:00 PM.⁶

The promotion of fast spindle frequencies during the day in younger subjects could represent one aspect of such a circadian waking signal, and the earlier reduction in spindle frequency in

the elderly could be related to an attenuation of this signal in the evening. Similarly, an earlier increase in spindle frequency at the end of the night could be associated with the often-reported difficulties for older people to maintain sleep in the morning hours.^{6,7} However, we did not find a clear correlation between spindle frequency and sleep consolidation in the majority of our subjects. Moreover, there is, to our knowledge, no experimental evidence that relates low spindle frequency to more-impaired sensory transmission, as compared with higher spindle frequencies. It remains to be elucidated if low-frequency spindles, resulting from longer hyperpolarization episodes, are particularly effective in reducing synaptic responsiveness.

Thus, our data do not provide evidence for a strong relationship between spindle frequency and sleep consolidation. An alternative interpretation would be that both the reduced amplitude in the circadian profile of sleep consolidation and the changes in the circadian modulation in spindle frequency are 2 different outputs of an attenuated circadian system in aging.

The circadian modulation of the other spindle variables was not greatly affected by age, except the less-pronounced nocturnal increase in spindle density in the older subjects. This finding is in contrast to results from a forced-desynchrony study, in which spindle frequency was the only parameter with a significant circadian modulation in older subjects, while younger subjects exhibited a clear circadian rhythm in spindle frequency, amplitude, density, and duration. Difference between the results from the forced-desynchrony study and the present nap study may, to some extent, come from the different imposed wake-sleep cycle in those studies (150 minutes/75 minutes in the nap study versus 18 hours 40 minutes/9 hours 20 minutes in the forced-desynchrony study). In accordance with previous reports,^{2,17-20} overall spindle density, amplitude, and duration were reduced in older subjects compared with the younger subjects. Lower spindle density and duration have been interpreted to be potentially based on age-related changes in thalamocortical and/or intracortical circuitries, such as an attenuation in the GABAergic mechanism involved in spindle generation.²⁰ The reduction in spindle amplitude and the increase in intraspindle frequency variability may reflect reduced synchronization in thalamocortical and cortical oscillations. This may also account for the generally reduced EEG power density in the entire frequency between 0.25 and 14 Hz with age.⁵

The effect of aging on spindle frequency is not unequivocal. Several studies, using visual or automatic spindle analyses, have reported an increase in mean spindle frequency with increasing age.^{2,17,19,20} Recently, automatic analysis of spindles in a large sample of subjects of different age groups has confirmed the decrease in density, amplitude, and duration but revealed a slight but significant reduction in spindle frequency with age.³⁸ A reduction of spindle frequency with age was also found in visually scored sleep spindles in an early study.³⁹ In the present study, we did not find any age-related frequency shift in sleep spindles during sleep in the naps. Naps may, however, be different from a consolidated night's sleep, since we found that spindle frequency tended to be higher in older than in younger subjects during the baseline night of this study.

In summary, the circadian rhythm of spindle frequency was attenuated and no longer phase locked with the circadian rhythm of melatonin secretion in older subjects. These changes were accompanied by a reduction of the sleep-propensity profile in the elderly. However, we did not find a correlation between spindle

frequency and sleep consolidation across the naps, and therefore, our data do not provide evidence for a relationship between spindle frequency and sleep consolidation.

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