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Age-related attenuation of the evening circadian arousal signal in humans

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Abstract

The human circadian pacemaker maintains timing and consolidation of sleep–wake behavior by opposing the build-up of homeostatic sleep pressure during the wake episode, particularly in the evening during the 'wake maintenance zone'. We tested whether age-related changes in sleep are a consequence of a weaker circadian arousal signal in the evening. Circadian rhythms and spectral components of the sleep EEG were investigated in 17 young (20–31 year) and 15 older (57–74 year) volunteers under constant posture conditions during a 40-h nap protocol (75/150 min sleep/wake schedule). Quantitative evidence for a weaker circadian arousal signal in aging arose from significantly more sleep occurring during the wake maintenance zone and higher subjective sleepiness ratings in the late afternoon and evening in the older group. In addition, we found a diminished melatonin secretion and a reduced circadian modulation of REM sleep together with less pronounced day–night differences in the lower alpha and spindle range of sleep EEG activity in the older group. Thus, our data indicate that age-related changes in sleep propensity are clearly related to a reduced circadian signal opposing the homeostatic drive for sleep.

Keywords: Aging; Wake maintenance zone; Sleepiness; Sleep–wake cycle; EEG spectral analysis; Melatonin

1. Introduction

The mammalian circadian system, one of whose major functions is the regulation of sleep and wakefulness, is governed by the hypothalamic suprachiasmatic nuclei (SCN) [52], for reviews see [53,72,74]. The differential neuronal activation of the SCN is dependent on various input signals from the environment such as light and social cues [34,40]. Lesions of the SCN eliminate circadian sleep–wake cycles in animals as well as circadian regulated endocrine functions, motor activity and core body temperature (CBT) rhythms [36]. The rare human lesion data support these findings [2,14,62]. How the SCN communicates within its sub-sections and with the rest of the brain and body to elicit physiological and behavioral function adequately synchronized to the appropriate time of day is still the subject of intense ongoing research [22,23,63].

The two process model of sleep and wakefulness predicts the day-to-day synchronization of an organism to its environment by the interaction of a circadian (C) and a homeostatic process (S) [3,21]. Two hallmarks of this system are remarkable: based on experimental findings and theoretical assumptions [26,47,68] a circadian arousal signal in the evening opposes the homeostatic drive for sleep shortly before melatonin onset and disappears prior to sleep onset (the 'wake maintenance zone'). The opposite occurs in the early morning shortly after the CBT nadir, when the homeostatic need for sleep is lowest and yet the circadian drive for sleep appears high. Therefore, the progressive increase in the circadian drive for sleep in the course of the nocturnal sleep episode counteracts the dissipation of homeostatic sleep pressure associated with consolidated sleep [26,47,68].

Evidence for this model comes from so-called forced desynchrony (FD) studies [16] where the volunteers' sleep–wake cycles are scheduled to artificial days longer or shorter than 24 h (e.g. 28 or 20 h). Under such conditions, the circadian pacemaker can no longer entrain to the imposed sleep–wake cycle and 'free-runs' with its own endogenous

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period. This means that sleep occurs at all different circadian phases, permitting assessment of the contribution of the circadian pacemaker and the sleep–wake dependent process to sleep propensity [20].

With age, some aspects of the sleep–wake cycle undergo well-known changes, such as shallower nocturnal sleep with more arousals, less slow wave sleep (SWS) and the prevalence of daytime naps [1,5]. Most [19,71,77], but not all studies [51,80] report a decline in the amplitude of circadian markers, e.g. melatonin, CBT and cortisol. The elderly usually [33,35], but not always [51] have earlier habitual bed- and wake-up times with an advanced circadian phase in relation to their CBT minimum (and melatonin secretion maximum), when compared to young volunteers. The endogenous circadian period remains rather stable with age [18]. It is not yet clear whether the circadian or homeostatic aspects of sleep regulation are more affected by age. We have recently obtained evidence that although the sleep homeostat retains its capacity to respond to sleep loss in aging, there is a significant age-related decrease of frontal delta predominance in this response [54]. Even though circadian facets of sleep regulation seem to be affected by age [28], we do not know whether aging per se causes changes in the circadian regulation of sleep and wakefulness, or whether they are rather a consequence of a modified regulation of circadian signaling downstream (or both). Moreover, to what extent the presumed dampened amplitude of circadian rhythms (such as melatonin, cortisol, CBT) is associated with changes in other physiological and neurobehavioral functions, has not been unambiguously clarified.

We, therefore, hypothesized both an age-related decrease in the circadian secretion of melatonin and a less pronounced day–night difference between sleep and wakefulness in the older group. This implies more sleep and higher subjective sleepiness levels during the day and less sleep and lower subjective sleepiness levels during the night.

We tested our hypotheses in a 40 h multiple nap protocol carried out under stringent constant posture conditions, with sleep and wakefulness occurring at different circadian times, using quantitative sleep EEG analysis along the anteroposterior axis, continuous assessment of subjective sleepiness and measurement of melatonin secretion.

2. Methods

2.1. Screening procedure

All study participants were recruited via advertisements at different Swiss universities and in newspapers. Only candidates with a Pittsburgh sleep quality index (PSQI) score ≤ 5 [6] and no extreme chronotype, i.e. ratings between 14 and 21 points on the morning-evening-type (M/E) questionnaire [69] were selected. Additionally, all potential study participants were asked about their sleep quality, life habits and health state. Exclusion criteria were: smoking, medication or drug consumption, shift work within the last 3 months and transmeridian flights within 1 month prior to the study. Each study volunteer underwent a physical examination, an interview, a neuropsychological test battery (only for the older group) and a polysomnographically recorded adaptation night. Only volunteers with a clinical sleep EEG scoring without any pathological findings [apnoea/hypopnoea-index (AHI) <10; periodic leg movements (PLM) <10/h] were included in the study. All participants gave written informed consent. The study protocol, the screening questionnaires and consent form were approved by the local Ethical Committee and conformed with the Declaration of Helsinki.

2.2. Study participants

Seventeen healthy young (nine females, eight males, age range 20–31 year, mean: 25.0 ± 3.3 S.D.) and 15 healthy older volunteers (seven females, eight males, age range 57–74 years, mean: 65.1 ± 5.6 S.D.) were selected for the study. The mean PSQI value was 2.1 ± 1.3 S.D. for the young and 3.4 ± 1.7 S.D. (t-test: $p < 0.05$) for the older volunteers. The ratings on the M/E questionnaire were slightly but significantly higher (earlier chronotype) in the older (mean 18.8 ± 3.0 S.D.) than in the young group (mean 16.4 ± 3.2 S.D.; t-test: $p < 0.05$). The mean body mass index (BMI) was 21.5 ± 1.6 (mean $\pm$ S.D.) for the young and 23.3 ± 2.1 for the older volunteers (t-test: $p < 0.05$). All subjects were free from medical, neurological, psychiatric and sleep disorders and were non-smokers without any drug abuse. The latter was verified for the young group by an urinary toxicological analysis, sensitive for amphetamines, benzodiazepines, opiates and tetrahydrocannabinol (Drug-Screen Card Multi-6®, von Minden GmbH, Moers, Germany). Except for five young female subjects taking oral contraceptives, none took any medication. Young females started the study on day 1–5 after menses onset during the follicular phase of their menstrual cycle.

2.3. Study design

One week prior to study begin (baseline week) participants were instructed to keep their individual bed- and wake times within a range of ± 30 min (compliance controlled by a wrist activity monitor, Cambridge Neurotechnologies®, Cambridge, UK and sleep logs). They were also required to abstain from excessive caffeine and alcohol consumption as well as heavy physical exercise. After the baseline week, the participants reported to the laboratory in the evening before day 1 (Fig. 1). The timing of their sleep–wake schedule was calculated such that the 8 h sleep episode was centered at the midpoint of each subject's habitual sleep episode as assessed by actigraphy and sleep logs during the baseline week. Habitual bedtimes did not vary significantly between groups (young: $23{:}34 \pm 56$ min versus older: $23{:}11 \pm 40$ min; mean $\pm$ S.D.; $p = 0.2$, t-test; mean difference: 23 min). The study started with an 8 h adaptation night in the laboratory. The following 16 h of wakefulness on day 1 were used

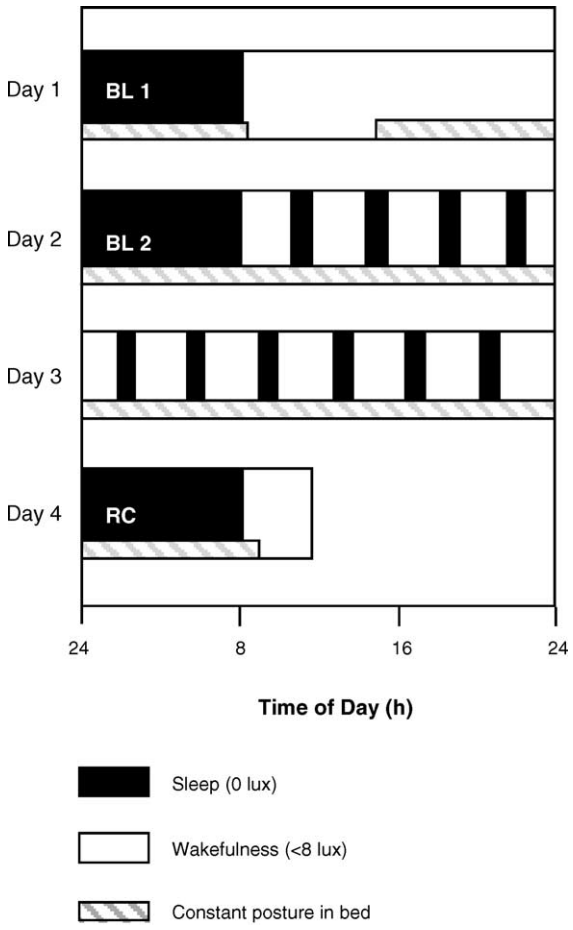

Fig. 1. Overview of the 4-day study protocol. Black bars (0 lux) indicate scheduled sleep episodes and white bars scheduled wake episodes (<8 lux). Hatched bars denote controlled posture (semi-recumbent during wakefulness and supine during sleep), BL = baseline night, RC = recovery night.

to adjust the subjects to the experimental dim light condition (<8 lux). During the morning a blood sample was taken from the older participants in order to verify both a normal haemogram and physiological coagulation. The older volunteers received a heparin low-dose injection on the three consecutive days of each study block (Fragmin® 0.2 ml, 2500 IE/Ul, Pharmacia AG, Dübendorf, Switzerland) in order to prevent any venous thrombosis. In the afternoon of day 1, all subjects were prepared for continuous polysomnographic recording. After a second 8 h sleep episode (baseline night), the subjects followed scheduled 75/150 min sleep–wake cycles during a 40 h protocol under constant conditions. This included constant recumbent body posture, no time cues, dim light conditions (<8 lux) during wakefulness and no light (0 lux) during sleep, and regular small isocaloric meals and water (for details of the method see [7,17]).

2.4. EEG sleep recordings and spectral analysis

Sleep was polysomnographically recorded with the VITAPORT ambulatory system (Vitaport-3 digital recorder, TEMEC Instruments B.V., Kerkrade, the Netherlands). Twelve EEGs, two electrooculograms, one submental electromyogram and one electrocardiogram were recorded. All signals were low-pass filtered at 30 Hz (fourth order Bessel type anti-aliasing, total 24 dB/October) at a time constant of 1.0 s. After online digitization by using a 12 bit AD converter (0.15 μV/bit) in the range of 610 μV and a sampling rate at 128 Hz for the EEG, the raw signals were stored on a Flash RAM Card (Viking, USA) and later downloaded to a PC hard drive. Sleep stages were visually scored per 20 s epochs (Vitaport Paperless Sleep Scoring Software) according to standard criteria [59].

EEG artifacts were detected by an automated artifact algorithm (CASA, 2000 PhyVision B.V., Gemert, the Netherlands). Spectral analysis was conducted using a Fast Fourier transformation (FFT; 10% cosine 4-s window) which yielded a 0.25 Hz bin resolution. All EEG power spectra were calculated during stage 2 in the frequency range from 0 to 32 Hz. Stage 2 was chosen because its duration did not significantly differ between night and day (see below). Finally, artifact free 4-s epochs were averaged over 20-s epochs. Here, we report EEG data derived from the midline (Fz, Cz, Pz, Oz) referenced against linked mastoids (A1, A2) in the range of 0.5–25 Hz.

2.5. Sleep stages

Visually scored sleep stages, NREM sleep (stages 2–4) and REM sleep were expressed as percentage of total sleep time (TST; stages 1–4 and REM sleep). Sleep efficiency (SE) and wakefulness after lights off (WALO) were expressed as percentages of nap time (time after lights off until time of lights on), whereas sleep latencies to stages 1 (SL1), 2 (SL2) or REM latency (RL) onset were indicated in minutes. Sleep stages were collapsed into 1.25 hourly bins per subject before averaging over subjects. In order to illustrate the dynamics within naps, TST of each group was binned into 5 min intervals. The time course of sleep stages within each age-group was analyzed with the Friedman-test, and group differences were calculated with the mean values over 11 naps (Mann–Whitney *U*-test).

2.6. Subjective sleepiness ratings

Subjective sleepiness was assessed on the Karolinska sleepiness scale (KSS) from 1 (very alert) to 9 (very sleepy) [37] every ∼30 min during scheduled wakefulness. Missing data were linearly interpolated. KSS values were collapsed into 1.25 hourly bins per subject before averaging over subjects. The very first sleepiness rating taken immediately after the naps was not included in this average in order to exclude sleep inertia effects [43].

2.7. Salivary melatonin

Saliva collections were scheduled during wakefulness at the same time intervals (every ∼30 min) as the subjective

Table 1
Melatonin analyses for young ($n = 17$) and older ($n = 15$) volunteers separately (mean ± S.D.)

Variable	Young (±S.D.)	Older (±S.D.)	p
Upward mean crossing time (h; clock time)	22.1 ± 1.0	22.0 ± 1.0	0.8
Downward mean crossing time (h; clock time)	7.9 ± 1.0	7.4 ± 1.0	0.1
Midpoint time of melatonin peak (h; clock time)	3.1 ± 0.8	2.7 ± 0.9	0.2
Mean 24 h secretion (between 5 and 29h; pg/ml)	9.1 ± 5.4	5.2 ± 2.5	<0.05
Mean secretion (between upward mean crossing time-downward mean crossing time; pg/ml)	18.9 ± 12.6	11.4 ± 6.1	<0.05
Mean duration (upward mean crossing time – downward mean crossing time; h)	9.8 ± 1.0	9.3 ± 0.6	0.1
Upward mean crossing time – wake time (h; elapsed time awake)	14.6 ± 0.7	14.8 ± 1.1	0.6
Downward mean crossing time – wake time (h; elapsed time awake)	24.4 ± 1.3	24.1 ± 1.0	0.5

sleepiness ratings (KSS). A direct double-antibody radioimmunoassay was used for the melatonin assay (validated by gas chromatography–mass spectroscopy with an analytical least detectable dose of 0.65 pm/ml; Bühlmann Laboratories, Schönenbuch, Switzerland; [75]). As for the sleepiness ratings, missing data were linearly interpolated and all melatonin values were collapsed into 1.25 hourly bins per subject before averaging over subjects. For calculating mean melatonin levels during the active secretion time, values of all samples between the upward- and downward-mean crossing points were averaged per subject and age group (see Table 1).

2.8. Classification of day and night naps

The first 75 min after lights off in the recovery night were considered as an additional nap. In order to compare EEG sleep spectra between 'night' and 'day' naps, the 24 h mean melatonin concentration (between hours 5 and 29 of the 40 h protocol) was calculated for each subject. This overall mean was 9.1 ± 5.4 pg/ml for the young and 5.2 ± 2.4 pg/ml (mean ± S.D.; t-test: $p < 0.05$) for the older group. A nap was rated as a night nap if the melatonin concentration of the last saliva sample before the nap was above the individual mean; otherwise, it was rated as a day nap. We defined the terms 'biological day' and 'biological night' according to this individual melatonin mean. The mean number of day and night naps per subject was 7.8 ± 0.8 (mean ± S.D.; day) and 3.2 ± 0.8 (night), for the young and 8.1 ± 0.8 (day) and 2.9 ± 0.8 (night), for the older volunteers and did not differ significantly between groups ($p > 0.1$). Only day and night naps containing a total stage 2 duration of at least 5 min were included in the EEG spectral analysis. The duration of stage 2 sleep within both age groups did not differ significantly between day and night naps [one-way rANOVA factor 'condition' (night nap versus day nap); $F_{1,16} = 0.2$, $p = 0.6$ for the young and $F_{1,14} = 1.3$, $p = 0.3$ for the older subjects] nor was there a significant interaction between the factors 'age' and 'biological day' versus 'biological night' (two-way rANOVA; $F_{1,30} = 0.49$; $p = 0.5$).

2.9. Statistics

For all analyses, the statistical packages SAS® (SAS Institute Inc., Cary, NC, USA; Version 6.12) and Statistica® (StatSoft Inc., 2000–2004, STATISTICA for Windows, Tulsa, OK, USA) were used. Mean values of visually scored sleep stages per nap sequence and KSS values were subjected to a Friedman-test for each age group separately with the repeated factor 'nap sequence'. A Mann–Whitney U-test was used for post-hoc comparisons since not all data reached the criterion for a normal distribution. Alpha adjustment for multiple comparisons was applied according to Curran-Everett [15]. For the correlation between KSS and sleep efficiency a Spearman rank correlation was used. For day–night comparisons, averaged EEG power density across biological daytime naps was compared with averaged values across biological nighttime naps. Two- and three-way rANOVAS with the factors 'age' (young and older), 'condition' (biological day and night) and 'derivation' (Fz, Cz, Pz, Oz) were performed. All p-values derived from rANOVAs were based on Huynh-Feldt's (H-F) corrected degrees of freedom (significance level: $p < 0.05$).

3. Results

3.1. Sleep stages during the naps

Sleep measures derived from visual scoring are summarized in Table 2. There were no significant age differences in TST, SE, WALO and stage 1 (Mann–Whitney U-test: $p > 0.1$). Older volunteers had significantly less SWS (stages 3 + 4) and REM sleep during the nap protocol than the young at the cost of significantly more stage 2, also reflected in more NREM sleep (p for all ≤ 0.006). SL1 revealed no significant age difference ($p = 0.98$), whereas RL was significantly shorter ($p < 0.001$) and SL 2 tended to be longer in young volunteers ($p = 0.07$). Post-hoc comparisons (Mann–Whitney U-test) revealed that the young participants slept significantly less during naps 4 and 10 and significantly more during naps 5 and 8 ($p < 0.036$; Fig. 2A). The same results were obtained for S.E. ($p < 0.036$; Fig. 2E) with a tendency in the latter during nap 7 ($p < 0.073$). This was also reflected in the duration of wakefulness (WALO) where older subjects had significantly less wakefulness during the wake maintenance zone (naps 4 and 10) and more during naps 5, 7 and 8 than the young ($p < 0.032$; graph not shown). Older subjects had more NREM sleep during naps 1, 4, 7 and 10 ($p < 0.036$; Fig. 2B). In parallel, the elderly had significantly more stage 2 (Fig. 2D)

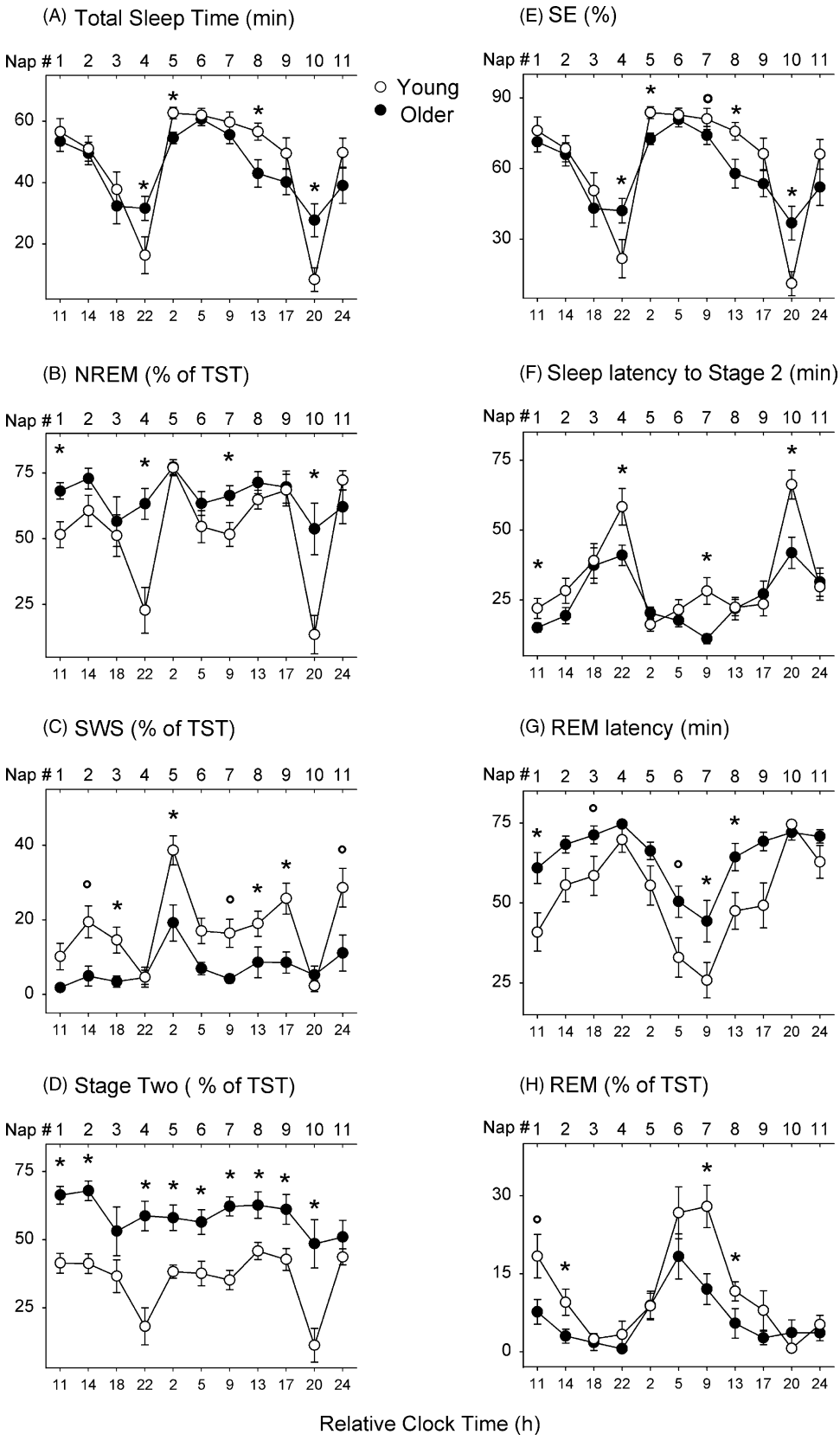

Fig. 2. Time course of sleep stages (2A–2H) across the 40-h nap protocol. Open circles: young volunteers ($n = 17$), filled circles: older volunteers ($n = 15$; mean ± S.E.M.), $^{*}p < 0.05$; $^{\circ}p < 0.1$.

Table 2
Sleep stages derived from visual scoring for both age groups, averaged across naps 1–11

Sleep variable	Young	Older	p	χ^2 young	χ^2 older
TST (min)	510.0 ± 19.6	487.3 ± 24.4	0.42	83.2 (**)	69.1 (**)
SE (%)	62.1 ± 2.4	59.0 ± 3.0	0.40	83.2 (**)	68.3 (**)
WALO (%)	37.5 ± 2.2	41.0 ± 3.0	0.34	85.3 (**)	68.3 (**)
Stage 1 (%)	26.3 ± 2.8	25.6 ± 2.4	0.84	17.1 (*)	17.6 (°)
Stage 2 (%)	35.6 ± 2.1	58.7 ± 3.1	<0.0001	35.3 (*)	16.8 (ns)
Stage 3 (%)	10.6 ± 0.7	6.0 ± 1.1	0.002	54.0 (**)	26.9 (*)
Stage 4 (%)	7.3 ± 1.2	1.2 ± 0.5	<0.0001	53.7 (**)	25.1 (*)
SWS (%)	17.9 ± 1.4	7.1 ± 1.4	<0.001	59.6 (**)	26.1 (*)
NREM sleep (%)	53.5 ± 2.1	65.8 ± 2.1	<0.001	56.7 (**)	11.5 (ns)
REM sleep (%)	11.1 ± 1.2	6.1 ± 1.0	0.006	78.5 (**)	49.1 (**)
SL1 (min)	18.3 ± 2.3	18.1 ± 2.1	0.98	79.0 (**)	87.4 (**)
SL2 (min)	32.3 ± 2.3	25.8 ± 2.3	0.07	66.6 (**)	80.1 (**)
RL (min)	52.1 ± 2.9	64.8 ± 1.7	<0.001	73.0 (**)	43.5 (**)

Values are indicated ±S.E.M., $n = 17$ for young and $n = 15$ for older subjects. TST = total sleep time (min; stages 1–4 + REM sleep); S.E. = sleep efficiency [%; (TST/time after lights off) × 100]; WALO = wake after lights off [%; (wakefulness + movement time)/time after lights off]; SWS = slow-wave sleep (% of TST; stage 3 + 4); NREM sleep = non-rapid eye movement sleep (% of TST; stage 2–4); SL1 (min) = sleep latency to stage 1; SL2 (min) = sleep latency to stage 2; RL (min) = latency to REM sleep (after sleep onset); p-values between age groups (fourth column; Mann–Whitney U-test) as well as in Chi-square (χ^2) and p-values from the Friedman-test for each group (d$F = 10$) are indicated (fifth and sixth column); °$p < 0.1$; ns, not significant.
* $p < 0.05$.
** $p < 0.001$.

during all naps ($p < 0.014$) except for naps 3 and 11. On the other hand, the young participants had more SWS (Fig. 2C) during naps 3 ($p < 0.036$), 5, 8 and 9 (with a tendency during naps 2, 7 and 11; $p < 0.073$) than the older subjects. SL2 (Fig. 2F) was shorter for the older group during nap 1 and during the wake maintenance zone (naps 4 and 10) as well as during nap 7 ($p < 0.036$). The significant longer SL2 in nap 7 for the young group could be explained with the very short RL in this nap. RL was longer for the older group during naps 1, 7, 8 ($p < 0.04$; with a tendency during naps 3 and 6, $p < 0.082$; Fig. 2G), which was exactly the time of day when most REM sleep (Fig. 2H) occurred. The older subjects had significantly less REM sleep during naps 2, 7, 8 ($p < 0.04$) and a tendency during the first nap ($p < 0.082$) although the circadian modulation of REM sleep was clearly present in both groups.

3.2. Time course of TST for young and older volunteers within naps

The time course of sleep within the naps and TST of each nap sequence is shown as a function of relative clock time in Fig. 3. There are sharp blue 'valleys' in the left panel which illustrate no or very little sleep for the young group at these specific time points in the evening (during naps 4 and 10). During the other naps, TST was relatively high in the young as indicated by the more long-wavelength colors (yellow and orange). The right hand panel of Fig. 3 illustrates the same

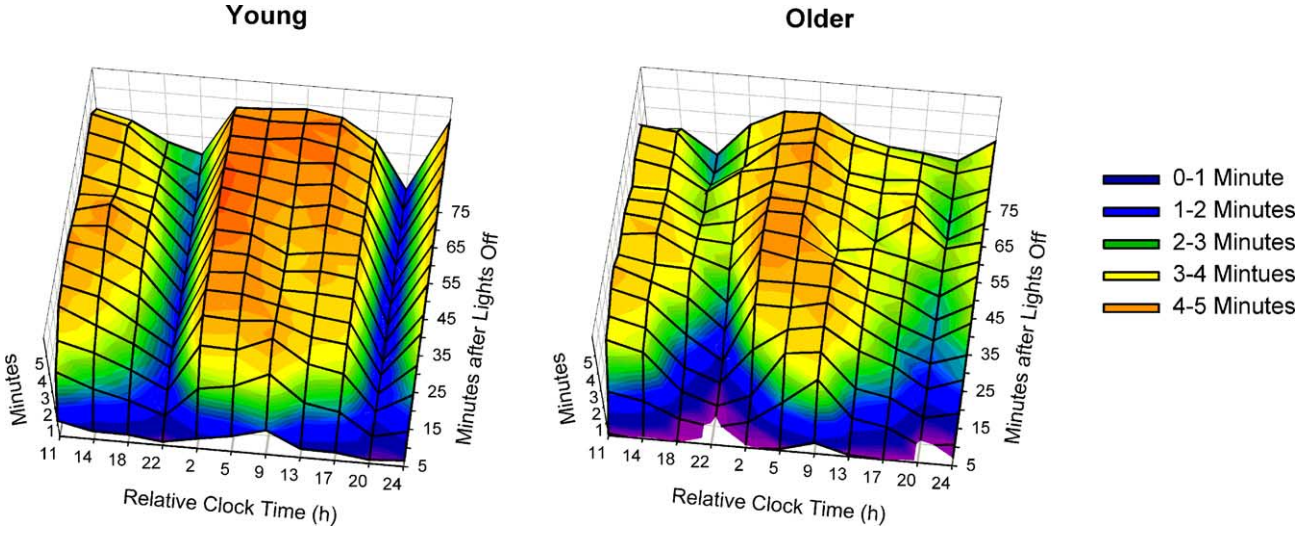

Fig. 3. Quasi three-dimensional plots of TST for both age groups. Left panel: young ($n = 17$) and right panel older subjects ($n = 15$). The x-axis represents the averaged mid-nap clock times for both age groups and the y-axis the time course within the respective naps (3–4 min). The z-axis specifies the amount of sleep (TST) per 5 min bin of each nap (min). Short-wavelength colors (blue, green) illustrate less sleep, longer wavelength colors (yellow, orange), more sleep.

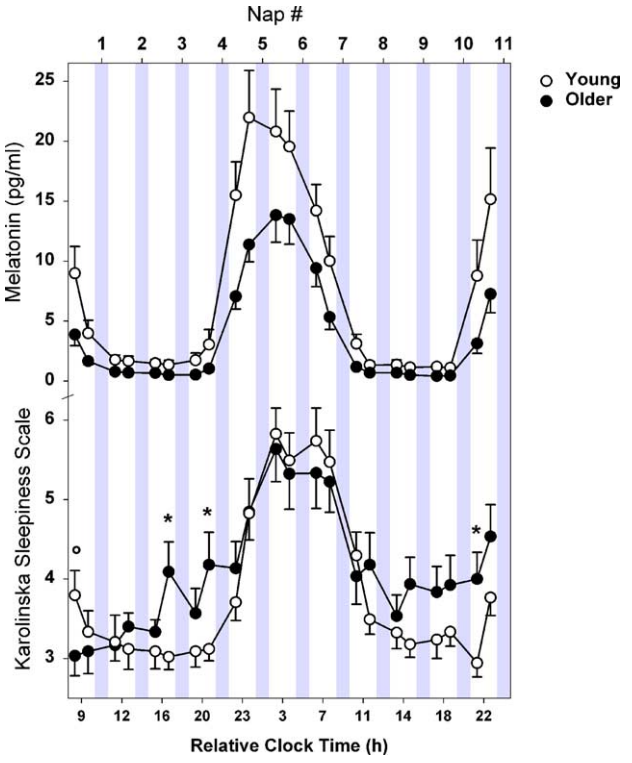

Fig. 4. The top panel shows the melatonin secretion during the 40-h nap protocol between young (white circles) and older volunteers (black circles, mean value + or – S.E.M. ($n = 17$ for the young and $n = 15$ for the older). The bottom panel represents subjective sleepiness ratings (KSS) of both age groups during scheduled wake episodes. ($^{*}p < 0.05$; $^{\circ}p < 0.1$).

three-dimensional interaction of relative clock time, minutes after lights off and the amount of TST per 5 min bin for the older volunteers. There was no significant difference in TST between both age groups, averaged over the entire 40-h nap protocol, but the lack of clear-cut blue valleys during the wake maintenance zone (naps 4 and 10) indicates that the older volunteers were able to sleep significantly more at this time of day even though SL was also longer in these naps (for statistics see Table 2). On the other hand, time intervals with much sleep (4–5 min, yellow and orange) were more scarce in the older than in the younger group, indicating a higher amount of wakefulness during naps outside the wake maintenance zone.

3.3. Time course of salivary melatonin, subjective sleepiness and sleep efficiency

The circadian rhythms of melatonin and subjective sleepiness (KSS) are illustrated in Fig. 4 (upper and lower panel). Older participants had a significant lower mean melatonin secretion (11.4 ± 6.1 older versus 18.9 ± 12.6 pg/ml young group; ±S.D., $p < 0.05$; *t*-test two-tailed for independent samples; see also Table 1). Moreover, a two-way rANOVA with the factors 'age' and 'nap sequence' yielded a main effect of age ($F_{1,30} = 6.9$; $p < 0.05$) and a tendency for the interaction of these factors ($p < 0.1$). Detailed measures of the timing, phase relationship and the mean secretion of melatonin are summarized in Table 1.

KSS values (lower panel) of both age groups exhibited a clear circadian modulation with highest sleepiness levels around the acrophase of their melatonin secretion. The time course of KSS ratings yielded a significant effect for each age group (Friedman-test: $p < 0.001$; $\chi^2 = 102.6$ for the older and $\chi^2 = 171.4$ for the younger group; d$F = 21$;). Older volunteers felt significantly sleepier in the late afternoon and evening of the first as well as in the evening of the second day (post-hoc comparisons: $p < 0.045$, Mann–Whitney *U*-test). The young subjects tended to feel sleepier after waking from a night's sleep (first wake episode $p < 0.09$). The mean sleepiness ratings averaged across all wake episodes did not significantly differ between groups. (3.8 ± 0.5 S.D. for the young and 4.1 ± 0.8 S.D. for the older volunteers; Mann–Whitney *U*-test: $p = 0.2$). The time course of subjective sleepiness differed from that of sleep efficiency (or total sleep time Fig. 2A). Correlation analyses between these two measures (i.e. the mean of each wake and adjacent sleep episode separately) revealed that the correlation coefficients were relatively low ($-0.2 < r < 0.3$) and not significant for all the naps (Spearman rank correlation).

3.4. Day–night differences in the EEG sleep spectra

The absolute mean of biological day- and night-spectra are illustrated in Fig. 5 (left and right hand panel) for both age groups. A significant interaction between the factors 'age', 'derivation' and 'condition' ($p < 0.05$, three-way rANOVA; performed on log-transformed data) occurred in the frequency range 0.75–2, 2.5–2.75, 3–3.25, 6.5–7, 7.25–8.75, 13.5.14 and 15–15.5 Hz. The day–night differences between both groups were significant in the frequency ranges: 7.75–8.0, 8.25–8.75, 11.0–14.0 and 14.5–15.75 Hz. Young volunteers developed an overall higher EEG density power during the biological day and night in the delta (0.5–4.5 Hz), theta (4.5–8.25 Hz), as well as in the spindle range (12.0–15.25 Hz) in all derivations (main effect of age; $p < 0.05$). Fig. 6 illustrates the EEG biological night spectra expressed as a percentage of the biological day spectra (=100%). A two-way rANOVA performed on relative EEG values (day/night ratio) of all derivations revealed a significant higher nocturnal EEG activity in the lower alpha (7.75–8.0 Hz, 8.25–8.75 Hz) and lower spindle range (11.0–14.0 Hz) in the younger group, whereas the higher spindle range (14.5–15.75 Hz) yielded significant higher relative values in the older group (main effect of age; $F > 4.6$, $p < 0.05$). These age differences were significant in all derivations and by visual inspection most pronounced in the parietal derivation. A significant interaction between the factors 'condition' and 'derivation' performed for each age group separately was found in the following frequency ranges for the young volunteers: 5.5–12.5, 12.75.0–14.75, 16.5–17.75, 18.5–19.0 Hz and for the older group between: 0.5–0.75, 1–1.25, 1.5–2.25,

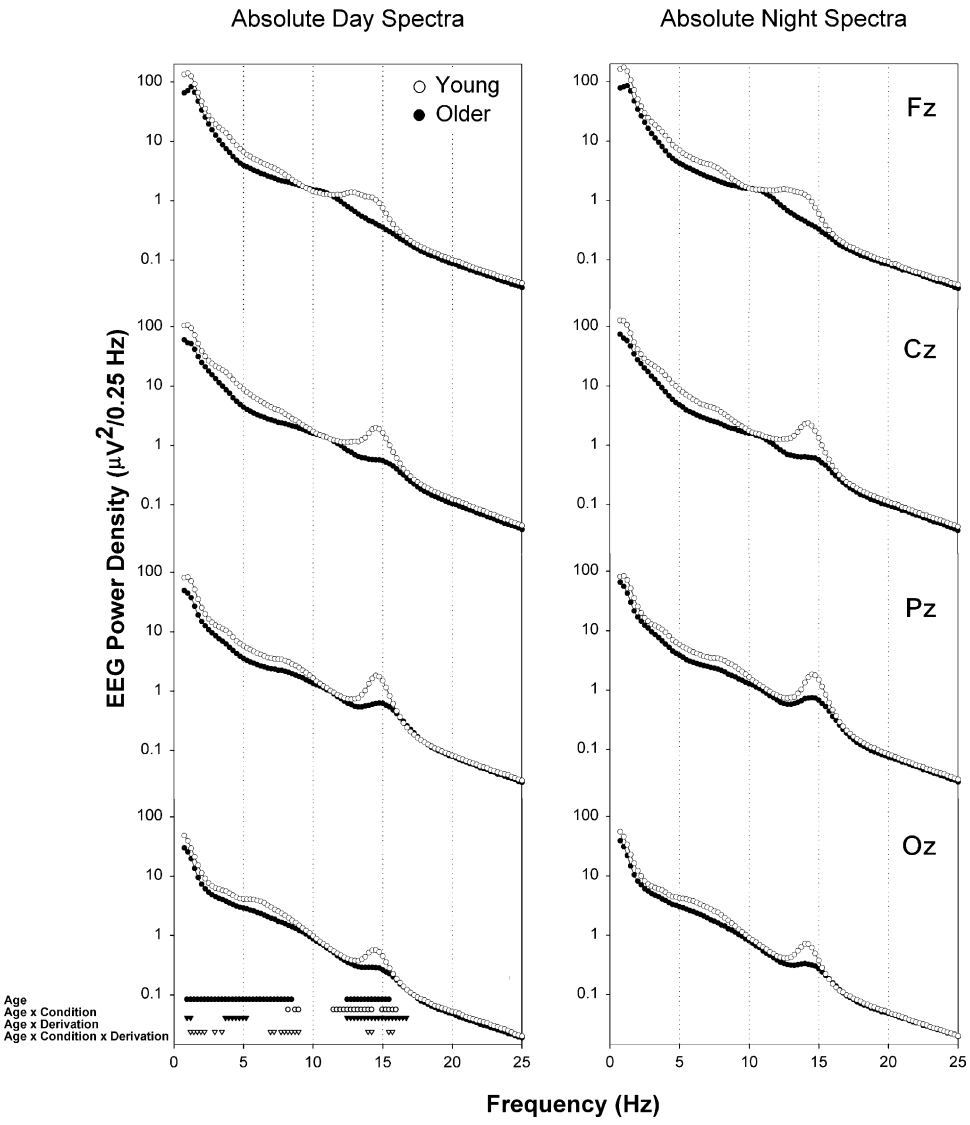

Fig. 5. Absolute biological day (left hand panel) and absolute biological night EEG sleep spectra during stage 2 (right hand panels) of young ($n = 17$; white circles) and older volunteers ($N = 15$; black circles) are shown in the frequency range between 0.5 and 25 Hz for Fz, Cz, Pz and Oz. Black circles near the abscissa indicate the frequency bins with significant age differences, the horizontal white circles show significant interaction between biological night and day for both age-groups and horizontal black triangles show significant interactions between age and derivation. For open triangles at the bottom the interaction 'age' × 'derivation' × 'condition' yielded significance ($p < 0.05$).

2.5–2.75, 10.75–11, 12.75–15.25, 17.25–18, 18.25–18.5, 18.75–19, 19.25–19.75, 20.0–21.25, 21.75–22.25, and 22.75–23.5, 23.75–24, 24.25–24.75 Hz (two-way rANOVA, p at least < 0.05).

4. Discussion

The study provides quantitative evidence for an age-dependent decrease of the circadian arousal signal in the evening. This is manifested in significantly more sleep in the older group during the wake maintenance zone. Furthermore, older subjects felt significantly more sleepy at circadian times corresponding to the late afternoon and evening (16:00–22:00 h). The study additionally confirmed the hypothesis of an age-related attenuation of melatonin secretion during the biological night in the healthy older group. Relative EEG power density during the biological night (percentage of daytime values) revealed a significant age-related reduction in the lower alpha (7.75–8.0 and 8.25–8.75 Hz) and in the lower spindle range (11.0–14.0 Hz), whereas the relative decrease in EEG power density in the higher spindle frequency range (14.5–15.75 Hz) was significantly less pronounced in the older group during the biological night.

4.1. Sleep stages

When the eleven scheduled 75 min naps were averaged, there were no age-related differences in sleep duration (TST) and sleep efficiency. However, the distribution of sleep stages

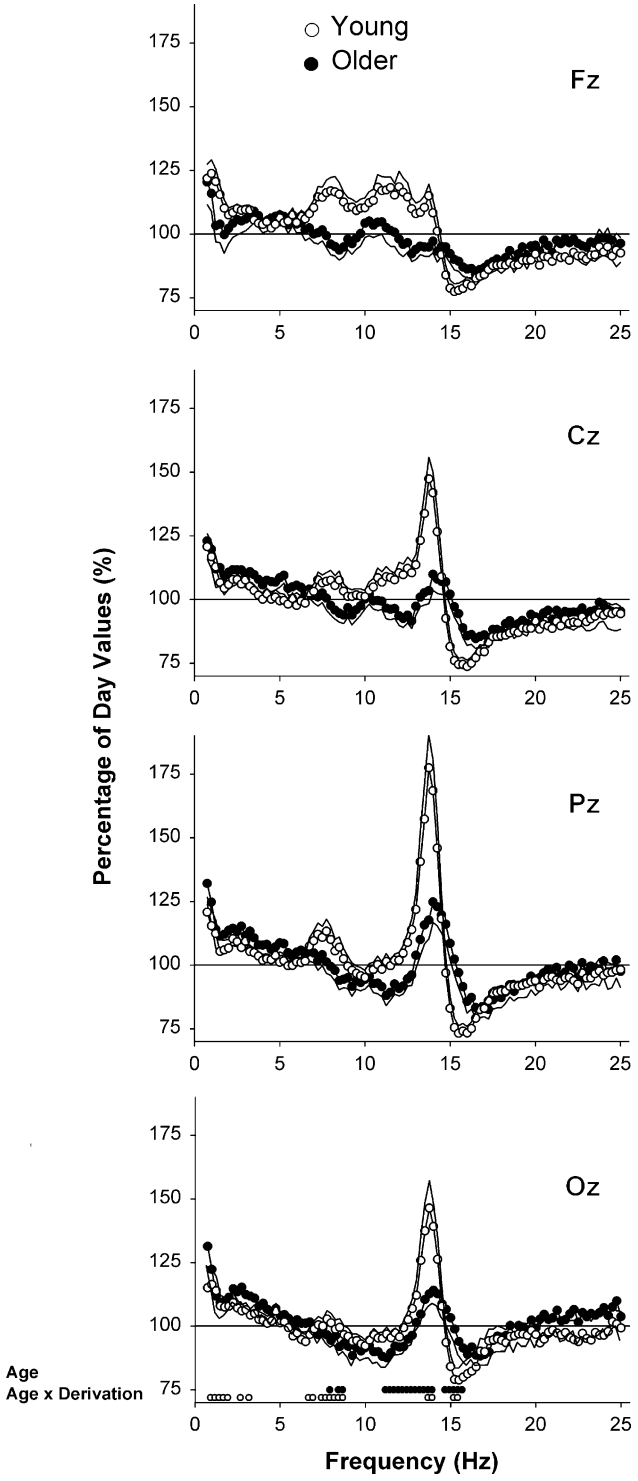

Fig. 6. Relative EEG spectra during the biological night (expressed as percentage of biological day values) are shown (all EEG spectra were analyzed during stage 2). Open circles indicate the young ($n = 17$; +S.E.M.) and filled circles the older subjects ($n = 15$; −S.E.M.). Circles near the abscissa specify the significant interactions between 'age' (black circles) and 'age' × 'derivation' (white circles), respectively ($p < 0.05$).

across and within sleep episodes was significantly altered by age, such that SWS and REMS was reduced in favor of stage 2. Our results corroborate findings from a nap-study with ultra short sleep–wake cycles (7/13 min) [38], whereby older subjects exhibited a higher sleep propensity (reflected in TST) during the 'wake maintenance zone'. Our young volunteers slept longer during several naps except for those during the wake maintenance zone, characterized by longer duration of wakefulness (WALO) and longer sleep latencies to stage 2 at this circadian time. On the other hand, neither WALO, nor sleep latency to stages 1 and 2 differed significantly between age groups. This is in contrast to FD studies, where older subjects slept less and were significantly longer awake during scheduled sleep at all circadian phases [28]. One possible explanation might be the age-related vulnerability to the desynchronizing effect of the FD protocol, (i.e. the problems of sleeping at adverse circadian times). This argument is further supported by simulated jet lag and shift work studies where older volunteers show a higher susceptibility to circadian phase misalignment [11,50]. Additionally, the higher amount of prior wakefulness (i.e. the wakefulness during scheduled sleep episodes and during the scheduled wakefulness) among the older volunteers in the FD protocol could have led to a modified proportion of sleep/wake cycles and therefore biased the duration and frequency of awakenings in those studies. In this sense, the multiple nap protocol has the advantage of being less masked by such evoked responses, because the frequency of scheduled sleep times was high (every 150 min) and the total duration of sleep episodes of 13.75 h was long enough to effectively 'counteract' the build-up of homeostatic sleep pressure.

The age-related reduction of SWS in our study was in accordance with many others [5,46] and clearly shows reduced NREM sleep intensity with aging. Interestingly, the portion of visually scored sleep stage 2 was significantly higher during all but the third and the last nap, which is at variance to other studies [28] where older subjects did not have more stage 2 sleep. The amplitude criterion of visual scoring (which according to Rechtschaffen and Kales [59] is confined to 75 μV for delta waves) might play a role in this difference, since older subjects tend to have lower EEG delta wave amplitudes. The significant difference between the age groups in stage 2 was presumably due to the fact that most young volunteers were not able to sleep during the wake maintenance zone. Based on the findings of Steriade et al. [67] (for a review see [66]), another possible interpretation of our data may be an age-related decrease in the hyperpolarized state of thalamocortical and cortical neurons and thus less synchronization and shorter periods of 'deep sleep' in favor of stage 2. Whether only the electrical potential is dampened with age or the number of neurons firing is reduced remains to be elucidated. A third interpretation for the SWS reduction with age might be a diminished homeostatic drive for sleep in the older group. According to the two process model of sleep regulation [3,21], SWS and SWA

depend exclusively on prior duration of wakefulness and exhibit age-related lower absolute levels [1], however, a full 'dose–response curve' with different levels of sleep pressure has not been carried out so far with aged subjects. A recent study has found that young and older adults manifest a similar homeostatic response to naps [10]. This is in accordance with previous [27] as well as our data [54] looking at the age-related changes in the homeostatic response after sleep deprivation.

Across the 40-h, the older group showed a shorter mean REM sleep duration, implying a diminished circadian rhythm of REM sleep compared to the young volunteers. Such age differences in mean REM duration have not been found in all studies [28,29] (for a review see [1]), even though a significantly shorter REM duration has been referred from a nap-study with ultra-short sleep–wake cycles [38]. Interestingly, mean RL was longer in our aged study group, which is in contrast to a FD study where significantly shorter mean RL for the older subjects was reported [28,29]. Thus, the duration of the imposed sleep–wake cycle may have contributed to this difference.

4.2. Subjective sleepiness

Older volunteers were significantly sleepier during the wake maintenance zone than the young. Whereas higher sleepiness in the older group began already during the first afternoon, it remained low in the young volunteers outside the melatonin secretion phase. Two interpretations are possible: first, the recuperative effect of napping during daytime to decrease homeostatic sleep pressure might be attenuated with age, resulting in higher accumulated sleepiness in the afternoon and evening. Second, the circadian arousal signal in the evening fails to adequately oppose increasing homeostatic sleep pressure in the older group. If the first argument is true, an age-related increase of homeostatic EEG markers during daytime, e.g. an increase of SWA in naps during the biological day and/or theta activity measured in the wake EEG should be observed in the older volunteers. However, there was no significant difference in SWA between the age groups during naps in the biological daytime. On the other hand, sleepiness and TST during the wake maintenance zone were higher in the older group, which corroborate the second argument. The time course of subjective sleepiness and sleep efficiency (or TST) were not correlated, indicating that subjective sleepiness and the ability to get enough sleep is not implicitly related.

A presumably different impact of the protocol on both age groups should also be taken into account, as spectral analysis of the wake EEG in the young subjects revealed that EEG low frequency components (1–7 Hz) during wakefulness, an index of homeostatic sleep pressure during wakefulness [8], were slightly enhanced on the second when compared with the first day of the nap protocol [8]. This suggests that even though theoretically sufficient sleep opportunities were presented, the fact that sleep was interrupted during the biological night (three naps of 75 min) may have led to a short-term enhancement of homeostatic sleep pressure. Clarification awaits the final analysis of EEG low frequency components during wakefulness in both age groups.

4.3. Melatonin secretion

Compared to the young volunteers, the mean melatonin secretion in our older group was decreased during the biological night, in accordance with several other studies [49,61,71] for reviews see [39,60]. The reason for this decline of hormonal secretion with age is unknown and not correlated with the size of the pineal organ [44].

It is well established that melatonin secretion is enormously different between individuals ('low secretor' versus 'high secretor'), which could be a reason why not all populations studied reveal such age differences [80]. When young and older subjects of that study with the lowest plasma melatonin values were binned together (e.g. the lower 15 percentile of each group), a significant reduction in the older group could be demonstrated in the 24-h average melatonin secretion and in the average nocturnal peak concentration [80]. Absolute levels of melatonin secretion do not correlate with sleep quality in the elderly [78] nor does administration of exogenous melatonin unambiguously improve sleep–wake behavior in healthy older people (for a review see [60]). On the other hand, there is an established association between the nocturnal 'sleep gate' and the onset of melatonin secretion [70] in younger subjects, shortly before habitual bedtime and immediately after the wake maintenance zone. When exogenous melatonin is administered in the late afternoon, the sleep time of young volunteers is advanced, permitting sleep even during the wake maintenance zone, which supports the tight association between melatonin onset and sleep gating [58]. From this, one could argue that changes in the timing of melatonin onset have repercussions on the timing of sleep [9]. Interestingly, the often reported age-related advanced sleep timing relative to circadian phase markers such as melatonin or CBT [32,33,35,79] was not found in our study nor by others [51]. We found no phase advance in the upward mean crossing time nor the midpoint of melatonin secretion, nor in the duration of secretion in the older age group; neither did the average bed- and wake-up times reveal significant differences between the age groups. Moreover, there were no significant age differences in the phase angles (e.g. melatonin upward- and downward mean crossing times since elapsed time awake). The only melatonin parameter which differed significantly was the lower mean melatonin secretion during the biological night (see above). Taken together, the altered age-related change in the sleep–wake pattern was presumably not determined by a phase advance in sleep–wake timing nor in shifts of the circadian phase marker (melatonin) in relation to the timing of sleep and wakefulness [the CBT analyses point in the same direction (unpublished data)].

4.4. Biological day night differences of the EEG spectra

Significant biological day–night differences between young and older volunteers were mainly found in the lower alpha and in the spindle range. Several studies have previously demonstrated an age-related reduction in EEG spindle activity [12,24,46,73,76]. The circadian modulation of sleep spindles [25] and the influence of exogenous melatonin during daytime in enhancing activity in the low spindle frequency range (13.75–14 Hz) and reducing activity in the high spindle frequency range (15.25–16.5 Hz) has been described in young subjects [30]. During the biological night (when endogenous melatonin is secreted) the peak in the EEG spindle range is (in young subjects) at a lower frequency range than during the biological day (outside the melatonin secretion window) [30,31,41]. These biological day–night shifts were found in both age groups of our study. However, in the older volunteers the nocturnal peak in the lower spindle frequency range was significantly attenuated and the nocturnal reduction in the higher spindle frequency range was significantly less pronounced, when compared to the young volunteers. The relative spectral differences in the spindle frequency range demonstrate a smaller shift between biological day and night spectra in the older group and were found in all derivations, but most pronounced in Pz. The supposed relationship between an age-related attenuated melatonin secretion and the altered activity in the nocturnal EEG spindle frequency range is not fully understood. The effects of exogenous melatonin on the sleep EEG are reminiscent of those induced by benzodiazepines, which act as $GABA_A$ agonists and enhance spindle generation (particularly in the low spindle frequency range [4]) in the nucleus reticularis of the thalamus [45].

Whether the circadian rhythms of sleep spindles are generated by the SCN directly through neuronal pathways [56] or indirectly via other pathways (or both) is not known. In rodents, direct projections from to the SCN to the thalamus (paraventricular nucleus) with highest neuronal activation during daytime have been recently reported [56]. Concomitantly, the SCN may project indirectly via the dorsomedial hypothalamus (DMH) to the VLPO [13] with most active neurons during sleep [65], for a review see [57]. The VLPO projects via GABAergic neurons to wake-promoting regions such as the histaminergic tuberomammillary nucleus and other monaminergic nuclei and has therefore a sleep-promoting effect [48,64].

A negative correlation between neuronal activity of the VLPO and the PVT seems likely to play a role in the regulation of sleep and wakefulness, at least in rodents [55,56]. Therefore, the significantly smaller day–night differences in the EEG spindle frequency range as well as in the alpha frequency range of our aged human study group might be due to an age-related attenuation of the circadian signal emanating from the SCN to the DMH and hence the VLPO, with consequently reduced inhibition of the brain stem ascending reticular activating system during biological nighttime. This may further result in higher arousability during sleep with less sensory inhibition of the thalamic nuclei, implying a reduced circadian modulation of sleep and wakefulness in the aging organism. More detailed analyses of the age-related changes in the EEG spindle range will be reported elsewhere [42].

Although no neurobiological substrate of the circadian arousal signal has been identified so far, our results confirm and extend previous findings that demonstrate age-related deteriorated output functions in this particular aspect of circadian sleep–wake behavior.

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