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.

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Keywords: Aging; Wake maintenance zone; Sleepiness; Sleep–wake cycle; EEG spectral analysis; Melatonin
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 antero-posterior 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 ± 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, benzo diazepines, 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 ± 56 min versus older: 23:11 ± 40 min, mean ± 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...
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 VI-TAPORT 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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young (±S.D.)</th>
<th>Older (±S.D.)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upward mean crossing time (h; clock time)</td>
<td>22.1 ± 10</td>
<td>22.0 ± 10</td>
<td>0.8</td>
</tr>
<tr>
<td>Downward mean crossing time (h; clock time)</td>
<td>7.9 ± 10</td>
<td>7.4 ± 10</td>
<td>0.1</td>
</tr>
<tr>
<td>Midpoint time of melatonin peak (h; clock time)</td>
<td>3.1 ± 0.8</td>
<td>2.7 ± 0.9</td>
<td>0.2</td>
</tr>
<tr>
<td>Mean 24 h secretion (between 5 and 29h; pg/ml)</td>
<td>5.4 ± 1.1</td>
<td>5.2 ± 2.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mean secretion (between upward mean crossing time-downward mean crossing time; pg/ml)</td>
<td>18.9 ± 12.6</td>
<td>11.4 ± 8.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mean duration (upward mean crossing time -- downward mean crossing time; h)</td>
<td>9.6 ± 1.0</td>
<td>9.3 ± 0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Upward mean crossing time – wake time (h; elapsed time awake)</td>
<td>14.6 ± 0.7</td>
<td>14.8 ± 1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Downward mean crossing time – wake time (h; elapsed time awake)</td>
<td>24.4 ± 1.3</td>
<td>24.1 ± 1.0</td>
<td>0.5</td>
</tr>
</tbody>
</table>
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., * \( p < 0.05 \); ◦ \( p < 0.1 \).
Table 2
Sleep stages derived from visual scoring for both age groups, averaged across naps 1–11

<table>
<thead>
<tr>
<th>Sleep variable</th>
<th>Young</th>
<th>Older</th>
<th>( p )</th>
<th>( \chi^2 ) young</th>
<th>( \chi^2 ) older</th>
</tr>
</thead>
<tbody>
<tr>
<td>TST (min)</td>
<td>510.0 ± 19.6</td>
<td>487.3 ± 24.4</td>
<td>0.42</td>
<td>83.2 (( * ))</td>
<td>69.1 (( * ))</td>
</tr>
<tr>
<td>SE (%)</td>
<td>62.1 ± 2.4</td>
<td>59.0 ± 3.0</td>
<td>0.40</td>
<td>83.2 (( * ))</td>
<td>68.3 (( * ))</td>
</tr>
<tr>
<td>Stage 1 (%)</td>
<td>26.3 ± 2.8</td>
<td>25.6 ± 2.4</td>
<td>0.84</td>
<td>17.1 (( * ))</td>
<td>17.6 (( * ))</td>
</tr>
<tr>
<td>Stage 2 (%)</td>
<td>35.6 ± 2.1</td>
<td>35.7 ± 3.1</td>
<td>&lt;0.0001</td>
<td>35.3 (( * ))</td>
<td>16.8 (( * ))</td>
</tr>
<tr>
<td>Stage 3 (%)</td>
<td>10.6 ± 0.7</td>
<td>6.0 ± 1.1</td>
<td>0.002</td>
<td>54.0 (( * ))</td>
<td>26.0 (( * ))</td>
</tr>
<tr>
<td>Stage 4 (%)</td>
<td>7.3 ± 1.2</td>
<td>1.2 ± 0.5</td>
<td>&lt;0.0001</td>
<td>53.7 (( * ))</td>
<td>25.1 (( * ))</td>
</tr>
<tr>
<td>SWS (%)</td>
<td>17.9 ± 1.4</td>
<td>7.1 ± 1.4</td>
<td>&lt;0.001</td>
<td>59.6 (( * ))</td>
<td>26.1 (( * ))</td>
</tr>
<tr>
<td>REM sleep (%)</td>
<td>53.5 ± 2.1</td>
<td>65.8 ± 2.1</td>
<td>&lt;0.001</td>
<td>58.7 (( * ))</td>
<td>41.3 (( * ))</td>
</tr>
<tr>
<td>SL1 (min)</td>
<td>11.1 ± 1.2</td>
<td>6.1 ± 1.0</td>
<td>0.006</td>
<td>78.5 (( * ))</td>
<td>49.0 (( * ))</td>
</tr>
<tr>
<td>SL2 (min)</td>
<td>32.3 ± 2.3</td>
<td>25.8 ± 2.3</td>
<td>0.07</td>
<td>66.6 (( * ))</td>
<td>80.1 (( ** ))</td>
</tr>
<tr>
<td>RL (min)</td>
<td>52.1 ± 2.9</td>
<td>64.8 ± 1.7</td>
<td>&lt;0.001</td>
<td>73.0 (( ** ))</td>
<td>45.5 (( * ))</td>
</tr>
</tbody>
</table>

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); SE = sleep efficiency [%; (TST/time after lights off) × 100]; W ALO = 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 (\( \chi^2 \)) and \( p \)-values from the Friedman-test for each group (\( d_F = 10 \)) are indicated (fifth and sixth column); \( * \)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...
Older participants had a significantly lower mean melatonin secretion during scheduled wake episodes. (*) p < 0.05, three-way rANOVA performed on log-transformed data) occurred in the frequency range 0.5–2, 2.5–2.75, 3–3.25, 6.5–7, 7.25–8.75, 13.5–15 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). Three-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; 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, 12.75–14.75, 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, 2.5–2.75, 3–3.25, 6.5–7, 7.25–8.75, 13.5–15 and 15–15.5 Hz. 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 0.5–2, 2.5–2.75, 3–3.25, 6.5–7, 7.25–8.75, 13.5–15 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.

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

The circadian rhythms of melatonin and subjective sleepiness 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 (F1,30 = 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; χ² = 102.6 for the older and χ² = 171.4 for the younger group; dF = 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

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 ± 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; **p < 0.1).
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. Further-
more, older subjects felt significantly more sleepy at circadi-
ann times corresponding to the late afternoon and evening 
(16:00–22:00h). The study additionally confirmed the hy-
pothesis of an age-related attenuation of melatonin secretion 
during the biological night in the healthy older group. Rel-
ative EEG power density during the biological night (per-
centage 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 rel-
ative decrease in EEG power density in the higher spindle 
frequency range (14.5–15.75 Hz) was significantly less pro-
nounced 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
opportunities were presented, the fact that sleep was inter-
suggests that even though theoretically sufficient sleep op-
comparably with the first day of the nap protocol [8]. This
ness, an index of homeostatic sleep pressure during wake-
EEG low frequency components (1–7 Hz) during wakeful-
alysis of the wake EEG in the young subjects revealed that
in 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 is true, an age-related increase of homeostatic EEG may have contributed to this difference.

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 [12,24,36,73,76]. 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 monoaminergic 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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