EEG Sleep Spectra in Older Adults Across All Circadian Phases During NREM Sleep

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Study Objectives: Healthy aging is associated with changes in sleep-wake regulation, and those changes often lead to problems sleeping, both during the night and during daytime. We aimed to examine the electroencephalographic (EEG) sleep spectra during non-rapid eye movement (NREM) sleep when sleep was scheduled at all times of day.

Design/Interventions: After three 24-h baseline (BL) days, participants were scheduled to live on 20-hour “days” consisting of 6.7 hours of bed rest and 13.3 hours of wakefulness for 12 consecutive days (forced desynchrony, FD). The EEG was recorded from a central derivation during all scheduled sleep episodes, with subsequent visual scoring and spectral analysis.

Setting: Intensive Physiological Monitoring Unit of the Brigham & Women’s Hospital General Clinical Research Center.

Participants: Twenty-four healthy older subjects (64.2 ± 6.3 yr; 13 women, 11 men)

Measurements and Results: Compared with BL nights, EEG activity in the slow wave (0.5 to 5.25 Hz), theta (6 to 6.25 and 7 Hz), alpha (10 to 11.25 Hz), and high spindle range (14.5 to 15.5 Hz) was significantly greater during FD, when subjects slept across many times of day and night. During FD, there was a significant interaction between homeostatic and circadian factors, such that EEG delta activity (0.5 to 1.5 Hz) was higher in the biological morning/early afternoon than at other times. EEG activity was significantly increased in almost all frequency ranges (0.5 to 21 Hz) during the biological day, as compared with the biological night, except for the lower EEG spindle range (12.25 to 14 Hz). Overall, EEG beta activity was positively correlated with wakefulness and negatively correlated with total sleep time.

Conclusion: Our findings provide some new evidence for the underlying mechanisms that contribute to age-related difficulties in sleep consolidation, especially when sleep occurs during the daytime.

Keywords: Aging, spectral analysis, 2-process model of sleep regulation

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HEALTHY AGING IMPACTS SLEEP DURATION, SLEEP CONSOLIDATION, AND SUBJECTIVE SLEEP QUALITY.1-3 SEVERAL STUDIES HAVE QUANTIFIED AGE-related changes in the human electroencephalogram (EEG) during sleep and have found a decrease in slow-wave activity (SWA; EEG activity in the frequency range between 0.75 and 4.5 Hz) with a shallower decline in SWA across the night4-6 and a decrease of EEG spindle activity, including spindle amplitude, incidence, frequency, and duration.7-9

The 2-process model of sleep-wake regulation, first described by Borbély and colleagues10 and established with data from young subjects, suggests that process S leads to a build-up of sleep pressure during wakefulness and shows an exponential decay during the following sleep episode. Process S is assessed experimentally by changes in the lower-frequency ranges (< 7 Hz) of the sleep and wake EEG. Thus far, the neurobiologic substrate of the homeostatic process S remains unknown, but several recent studies have provided evidence that genetic factors, neuronally active molecules, or both are associated with homeostatic sleep regulation.11-14

Process C in the 2-process model describes a circadian rhythm of sleep propensity, driven by the circadian timekeeping system with its master clock in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus.15 The 2-process model of sleep regulation suggests that the circadian sleep propensity rhythm (process C) counteracts the homeostatic system (process S) to achieve consolidated wakefulness and sleep in humans. This balance is achieved by a progressively stronger wake-promoting signal from the biological clock across the habitual waking day, becoming strongest in the late evening shortly before habitual bedtime (the so-called “wake-maintenance zone” or “forbidden zone for sleep”16-18). This circadian wake-promoting signal counteracts the increasing level of sleep pressure that accumulates over the course of the waking day. During sleep, the 2-process model implies a sleep-promoting signal from the biological clock that increases in strength toward the end of the night, allowing continuation of sleep as homeostatic sleep pressure is dissipated.17-19

It has been hypothesized that the strength of 1 or both processes (S and C) might weaken with age, thereby affecting sleep. If process C were to undergo an age-dependent decline, both the SCN and its downstream output variables could be affected. Indirect evidence for this includes reports of reduced numbers of SCN cells in postmortem human brains from older adults.20-21 Additional evidence for an age-related decline of signals from the circadian system comes from lower amplitudes of physiologic circadian rhythms in humans, including the rhythm of core body temperature22-24 and melatonin,25,26 although a well-known wide inter-individual variability exists among aged individuals.27,28 Furthermore, the phase relationship between the timing of sleep-wakefulness and the timing of circadian rhythms of melatonin and core body temperature changes with age, for reasons that are not fully understood.29 Data from 2

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multiple nap protocols, which keep homeostatic sleep pressure low by scheduling subjects to a very short sleep-wake cycle (7/13 min, 30/60 min, or 75/105 min) across several days, found more sleep during the evening “wake maintenance zone” in older participants, compared with young adults, suggesting a change in the circadian promotion of wakefulness at that time of day.

There is also evidence that the homeostatic process undergoes age-related changes. Support for this includes results from animal studies, which have found age-dependent cell loss in the ventrolateral preoptic nucleus (VLPO) with its sleep-promoting (GABAergic) and galaninergic neurons. In humans, the volume and cell number in the VLPO area has also been reported to decrease with age. Thus, a decline in VLPO activity could contribute to age-related changes in homeostatic sleep pressure in humans. In addition, as noted above, in humans there are age-related reductions in SWA, an aspect of the EEG that, in young adults, is used as a marker of process S.

The regulation of EEG sleep spindles is under both homeostatic and circadian control, and it has been shown that EEG slow spindles, generated in the thalamus, prevent cortical brain areas from receiving sensory inputs during sleep. In young subjects, EEG activity in the low spindle range follows a clear circadian pattern, which is 180° out of phase with activity in the high spindle range, such that the greatest EEG activity in the low spindle range occurs mainly during the nighttime when endogenous melatonin is present. In older subjects, the incidence, duration, and amplitude of sleep spindles is attenuated, as shown in a prior forced-desynchrony (FD) study from our laboratory. The finding that EEG activity in the spindle range is affected by aging is supported by a study using a multiple-nap protocol, which found that, when daytime sleep was compared with nighttime sleep, the EEG sleep spectra differed between young and older subjects mainly in the EEG spindle range, with lower day-night differences and lower EEG spindle frequency in the older subjects. Prior reports have also demonstrated that, while the circadian modulation of spindle frequency is phase locked with the circadian rhythm of melatonin secretion in young adults, there is a weaker coupling between spindles and melatonin in older subjects.

Under normal entrained conditions, the 2 sleep-wake regulatory processes have a fixed relationship to each other and are varying simultaneously. FD protocols have been used to study the relative contribution of the homeostatic and circadian processes—and their interaction—to sleep regulation in humans. In FD protocols, subjects are scheduled to live for days or weeks on a sleep-wake cycle that is several hours shorter or longer than the near-24-hour period of the circadian system (in the studies above, 28 hours). As a result, sleep and wake episodes are scheduled across all circadian phases. The endogenous circadian pacemaker cannot entrain to this imposed sleep-wake cycle and oscillates at its near-24-hour period, thus allowing separation of circadian-dependent and homeostatic sleep-dependent influences on the data. In a prior 28-hour FD study conducted in our laboratory, visually scored sleep stages of young and older subjects were compared. We found that, at all circadian phases, the older subjects slept for a shorter duration than did young subjects and, when the latter part of sleep occurred at adverse circadian phases, the sleep of the older subjects was disrupted to a greater extent than that of the young subjects. Our detailed analyses of those data suggest that the age-related reduction of sleep consolidation is primarily due to a reduction in the consolidation of non-rapid eye movement (NREM) sleep.

Although multiple-nap protocols have examined EEG sleep spectra during sleep scheduled across circadian phases, the design of those protocols was such that homeostatic sleep pressure could not build to very high levels. It remains to be elucidated what the circadian and homeostatic impacts on the EEG sleep spectra are in older people when sleep pressure is allowed to accumulate to a higher level (close to that of a “normal” 16-hour hour waking day). An advantage of FD protocols over nap protocols is that they offer the possibility to assess the dynamics of homeostatic sleep pressure over a “normal” sleep duration at all circadian phases. Therefore, in the present analysis, we aimed to quantify the EEG sleep spectra during NREM sleep in healthy older subjects when sleep was scheduled across all circadian phases to better understand EEG factors that might contribute to differences in sleep consolidation related to circadian phase.

In a previous FD study performed in our laboratory, we showed lower sleep consolidation in older subjects at all circadian phases, when compared with a young subject cohort. Therefore, in the present analysis, we hypothesized that there would be lower sleep consolidation during the biological daytime when compared with sleep occurring during the biological nighttime or when compared with baseline sleep. Secondly, given earlier reports from our FD study and from nap studies, which applied methods for a detailed characterization of EEG sleep spindles, we also expected to confirm that the EEG spindle range would be impacted by circadian phase. Finally, because analysis of EEG sleep spectra across a broad frequency range has not been reported previously for EEG data collected in an FD study in older adults, we aimed to perform such an analysis on our dataset to determine how the EEG spectra is impacted by sleep of approximately normal duration scheduled at different circadian phases.

SUBJECTS AND STUDY DESIGN

Subjects

Twenty-four (13 women, 11 men) healthy older subjects participated in the study (age range: 55–78 years; mean ± SD: 64.2 ± 6.3 years), with the women slightly younger than the men (women: 61.9 ± 5.1 years; men: 66.9 ± 6.7 years; P = 0.051). The habitual bedtimes of the subjects were, on average, 22:54 ± 44 minutes.

Prior to their study, all participants underwent a screening process to ensure that they were healthy and free from medical, psychiatric, and significant sleep disorders. This included a physical examination, routine biochemical blood and urine tests, an electrocardiogram, psychological questionnaires (the Minnesota Multiphasic Personality Inventory, the Geriatric Depression Scale, and the Folstein Mini-Mental State Examination), and an evaluation by a clinical psychologist. Body mass index was used as an exclusion criterion and had to be greater than 18 kg/m² and less than 30 kg/m². In addition, all subjects had an overnight clinical polysomnographic recording to screen out those with significant sleep disorders (apnea index > 10 or
periodic leg movement with arousal index > 15). The study procedures were reviewed and approved by the Human Research Committee of Partners Health Care System and conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent for both the screening and the inpatient protocol and were reimbursed for their study participation.

Study Design
For the 3 weeks prior to the 32-day inpatient study, subjects were instructed to maintain a regular sleep-wake schedule with 8 hours time in bed at a self-selected target time within a range of ± 30 minutes. Compliance was verified for at least the final week by means of a wrist activity monitor (Actiwatch®, Phillips Respironics, Murrysville, PA), sleep diaries, and telephoning to a time-stamped call-in line each night before retiring and each morning after arising. The subjects were instructed to abstain from all medications, caffeine, nicotine, and alcohol during these 3 weeks and throughout the inpatient study. Compliance with this was verified by a toxicologic urine test during screening and upon admission to the laboratory. The inpatient study began with three 24-hour baseline (BL) days with 16 hours of wakefulness and 8 hours of bedrest, scheduled at each subject’s average bedtimes from the last 7 days of screening. These BL days were followed by thirty 20-hour days of FD, each consisting of 13.3 hours of wake and 6.7 hours of scheduled bedrest (Figure 1). This resulted in sleep and wake episodes occurring 4 hours earlier each day and occurring across all circadian phases. Three 24-hour recovery days followed the FD protocol.

The original study was designed to test the effects of presleep administration of 2 doses of exogenous melatonin in a double-blind, counter-balanced, cross-over design. This resulted in 4 groups. On every night of the study (including the BL nights), subjects were given pills 30 minutes prior to scheduled lights out. Thirteen subjects received melatonin for their first consecutive 12 FD sleep episodes, whereas 11 subjects received placebo first and melatonin for their final 12 FD sleep episodes. After the first 12 FD sleep episodes, there was a “washout” for 6 sleep episodes, when all subjects received placebo (Figure 1). Here, we report only results from the second and third BL nights and the 12 FD sleep episodes during each subject’s placebo condition.

During the study, each subject lived in a private room without information about time of day in the Intensive Physiological Monitoring Unit at the Brigham and Women’s Hospital General Clinical Research Center. Room temperature was kept at approximately 24°C, and ambient light levels during wake episodes were less than 0.0087 W/m² (~3.3 lux) at 137 cm from the floor facing the walls and had a maximum of 0.048 W/m² (15 lux) at 187 cm from the floor facing the ceiling. During scheduled sleep episodes, the lights were turned off. The subjects received regular meals and were not allowed to sleep, lie down, or nap during scheduled wake episodes.

METHODS

Core Body Temperature
Core body temperature (CBT) was collected at 1-minute intervals throughout the study using a rectal thermistor (Measurements Specialties, Inc., Hampton VA) to assess circadian period and phase for each subject. Circadian period was assessed by performing nonorthogonal spectral analysis on the CBT data from the placebo condition. This method takes into account the imposed 20-hour rest-activity cycle and then searches for an unknown periodicity within the circadian range (search range used in this study was 15-30 hours), using an exact maximum-likelihood fitting procedure. Using the period and the projection of the CBT minimum on the first day of FD during the placebo condition (assigned circadian phase 0°), we then assigned a circadian phase from 0° to 359° to each minute of the FD segment of the study and used this to assign each epoch of sleep a circadian phase.

Plasma Melatonin
Plasma samples were collected approximately every 60 minutes throughout most of the BL segment of the study via an indwelling venous catheter. Each plasma sample was frozen and assayed after study, in either the Brigham & Women’s Hospital General Clinical Research Center Core Laboratory or at Pharmasan Labs (Osceola, WI).

We assessed dim-light melatonin onset (DLMOn) and offset (DLMOff) during the BL (when the subjects were sleeping at their habitual times) and applied this timing information to the FD to classify all segments of the FD as biological night (times when endogenous melatonin was present) or biological day (times without endogenous melatonin secretion). We used 2 different methods to do this (for a review see©) to account for the fact that the exact time of transition between biological day and night is difficult to determine. In the first method, we...
fitted each subject’s melatonin data from a 24-hour BL day with a 3-harmonic regression model to determine the amplitude of the fitted melatonin peak. We then used linear interpolation to calculate the time at which the melatonin values crossed 25% of this fitted amplitude. These times are referred to as the DLMO and DLMOff. In the second method, we determined the times during BL at which melatonin secretion was greater than 2 SD of the daytime values and times at which it was less than 2 SD of the BL daytime values.

The time between the 25% DLMO and DLMOff was assigned as biological night, and the time when plasma melatonin levels were below 2 SD of the baseline daytime values was assigned as biological day (no or very low melatonin present) (Figure 2). There was a small zone at the transition between biological day and night when very little melatonin was present, and we included this zone in our biological day analysis. The times at the transitions between biological day and night overlapped by 62 ± 8 minutes (mean ± SD) at melatonin onset and 105 ± 24 minutes at melatonin offset across all subjects (see Figure 2). Due to technical problems collecting blood samples from 1 subject during the BL, this subject was excluded from the DLMO analysis.

**EEG Recording and Spectral Analysis**

The EEG was recorded during all sleep episodes using a standard montage (C3, C4, O1, O2), referenced to contralateral mastoids (A1, A2). In addition to the EEG, 2 electrooculograms (left outer canthus, right outer canthus), 1 submental electro-EMG, and a 2-lead electrocardiogram were recorded. All signals were acquired using a digital ambulatory sleep recording system (Vitascore, version 1.40, Temec Instruments, Kerkrade, B.V., The Netherlands). The EEG signals were high-pass filtered at a time constant of 0.68 seconds and low-pass filtered at 70 Hz (Bessel fourth-order antialiasing; > 80 dB). Finally, the signals were digitized with a resolution of 12 bit (range 500 µV; sampling rate 256 Hz, storage rate 128 Hz), stored on a Flash RAM card, and downloaded offline after wake time.

All sleep episodes were scored visually according to standard criteria by trained scorers who were blind to the study conditions. After artifacts had been manually removed, all 30-second epochs scored as NREM sleep were subjected to spectral analysis (fast Fourier transformation, 4-s window), which resulted in a 0.25 Hz resolution (Vitascore, version 1.40, Temec Instruments). For data reduction, artifact-free 4-second epochs were averaged over 30-second epochs. EEG power spectra were calculated during NREM sleep (sleep stages 2-4) in the frequency range from 0 to 32 Hz. We report here data from the central derivation (preferably C3, but, when C3 was not available, C4 was used) in the frequency range between 0.5 and 25 Hz. We excluded 9 sleep episodes that were missing more than 60 minutes of scorable EEG data, were comprised of more than 60 minutes of artifacts during sleep, or were of bad signal quality. For 1 subject, posthoc analysis of the plasma data suggested that the subject inadvertently received melatonin on 2 of the nights he was supposed to have received placebo; these 2 sleep episodes were excluded from analysis. This resulted in a total of 323 sleep episodes included in the analysis.

We defined sleep latency (SL) as the time from lights off until the occurrence of any stage of sleep, whereas sleep offset (SOFF) was defined as the duration from the final epoch of any stage of sleep until lights on. Analyses performed on SL and SOFF were done on log-transformed data because the data lacked a normal distribution. For sleep-stage data, NREM sleep (stages 2-4), rapid-eye movement (REM) sleep, slow wave sleep (SWS; stage 3 and 4), and stages 1-4 were calculated in minutes and were also expressed as a percentage of total sleep time (TST) for the BL (8-hour) and the FD (6.7-hour) sleep episodes. Wakefulness during scheduled sleep and sleep efficiency (SE) were calculated as the percentage of total time in bed between lights off and lights on.

The EEG sleep spectra from the FD condition (in which sleep occurred across all circadian phases) were first compared with those from the BL nights. To do this, all 6.7-hour FD
sleep episodes were averaged together and expressed as a percentage of the first 6.7 hours of the BL nights (so as to compare the same duration in BL and FD sleep episodes).

To examine circadian and sleep-dependent influences on the EEG sleep stages and spectra, each 30-second epoch of sleep was assigned both a circadian phase and a homeostatic time-within-scheduled-sleep interval. For circadian phase, each epoch was allocated to a circadian phase between 0° and 359° (relative to the CBT minimum at 0°; see above). The data were then averaged into 45° circadian bins (equivalent to 3 circadian hours) referred to as “circadian phase.” For the assessment of the homeostatic sleep-dependent influence on EEG sleep stages and spectra, every epoch was also binned into 1 of 4 quarters with respect to elapsed time since lights out, which resulted in four 1.675-hour “homeostatic intervals.”

We also determined whether each sleep episode during the FD segment occurred during biological day or biological night. Biological night included those sleep episodes that occurred at the same circadian phases as between the BL 25% DLMOn and DLMOff. Biological day included those sleep episodes at the same circadian phases in which BL melatonin levels were lower than 2 SD of the daytime values, as well as those in the transition zone, when levels were above 2 SD of daytime values but below 25% DLMOn values. Only sleep episodes that occurred entirely (±10 minutes) within biological day or biological night (based on the above definitions) were used for this analysis, which included 126 FD sleep episodes. In this analysis, biological day or biological night sleep episodes were expressed as a percentage of the first 6.7 hours of BL.

Statistical Analysis
All statistical analyses were performed with SAS (SAS Institute Inc, Cary, NC, Version 9.1) or Statistica (Stat Soft Inc., 1984-2004, STATISTICA for Windows, Tulsa, OK). EEG stages and spectra were expressed relative to BL means (mean of the second and third BL night), or z-transformed. Repeated 1-, 2-, or 3-way analyses of variance (rANOVA) were performed either on standardized (z-scores) or log-transformed data with the independent variables “condition” (BL, FD), “biological time” (biological day, biological night), “order” (placebo in the first or second half of the protocol), and “sex.” All P values derived from rANOVA were based on Huynh-Feldt corrected degrees of freedom. For analyses with the factors “homeostatic interval” (i.e., time intervals since sleep onset) and “circadian phase” (i.e., 45° [~3-h] bins), a mixed-model analysis was performed due to unequal sample sizes; those P values were based on Kenward-Roger corrected degrees of freedom. Post hoc comparisons were performed by using Duncan multiple range tests or by applying F tests on the least-square means and a modified Tukey HSD test. P values from repeated measurements were adjusted. For correlational analyses, EEG sleep stages and EEG spectral activity were binned into circadian phases for each subject (as described above). Correlation analyses (Pearson correlation) between EEG sleep stages and EEG spectra were then performed on standardized data (i.e., deviations from mean) across all circadian phases and for all subjects. In a second step, we also correlated EEG sleep stages and EEG spectra for each circadian phase separately (i.e per 45° [~3-h] bin).

RESULTS

Sleep Stages
The duration of NREM, REM, stage 1, stage 2, and overall TST were significantly shorter in FD than during BL, whereas the duration of wake was significantly longer (see Table 1). Because of the shorter scheduled sleep episode in FD, we also examined the percentage of each stage and found that SWS and wake were a significantly greater percentage of TST, whereas stage 2 and SE were significantly reduced (see Table 1). Women spent significantly more time in NREM sleep with shorter times awake and a shorter SL than men (P < 0.05; main effect of sex). The order of placebo administration did not have a significant effect on any of the sleep-stage variables (1-way analysis of variance; P > 0.8).

Wakefulness and TST showed a significant circadian and homeostatic variation (P < 0.05; main effects of circadian phase and homeostatic interval; see Figure 3) as well as a significant interaction (P < 0.05; 2-way rANOVA). Posthoc analysis revealed a significant increase of wakefulness in the second half of scheduled sleep episodes (homeostatic intervals 3 and 4) when that portion of the scheduled sleep episode occurred at circadian phases between 135° and 270°, which correspond to the afternoon and evening (P < 0.038). TST was significantly reduced when the end of the scheduled sleep episode occurred at circadian phases between 90-270° (corresponding to the biological daytime; P < 0.044). SWS exhibited a significant decrease across the scheduled sleep episodes (P < 0.05; main effect of homeostatic interval) with no significant circadian variation or interaction between the 2 factors.

Table 1—Sleep stages during baseline and forced desynchrony

| Sleep stage | BL, min | FD, min | BL, % | FD, % | Sex (Fp) |
|-------------|--------|--------|-------|-------|----------|
| NREM        | 234.1 ± 35.6 | 177.9 ± 25.4 | 62.7 ± 6.3 | 63.7 ± 5.7 | (5.9 min) |
| REM         | 85.7 ± 19.9 | 66.6 ± 14.0 | 22.9 ± 4.8 | 23.1 ± 3.8 |
| SWS         | 49.7 ± 33.9 | 49.4 ± 21.3 | 13.6 ± 9.1 | 18.0 ± 7.5 |
| Stage 1     | 53.0 ± 27.0 | 36.1 ± 18.1 | 14.3 ± 7.1 | 13.3 ± 6.7 |
| Stage 2     | 184.5 ± 39.4 | 124.6 ± 23.8 | 49.2 ± 9.1 | 45.7 ± 7.3 |
| Wake        | 99.0 ± 35.9 | 118.9 ± 32.1 | 20.7 ± 7.5 | 29.8 ± 8.0 |
| TST         | 372.8 ± 39.1 | 280.5 ± 32.0 | 78.0 ± 8.4 | 70.3 ± 8.0 |
| SE          | -       | -       | 78.0 ± 8.4 | 70.3 ± 8.0 |
| SL          | 7.6 ± 7.1 | 6.4 ± 3.4 | -       | (6.4) min |
| SOFF        | 17.0 ± 41.2 | 27.2 ± 20.2 | -       | -       |

Notes: Sleep stages (mean ± SD; N = 24) as a percentage of total sleep time (TST) during the baseline (BL) and the forced-desynchrony (FD) conditions. Duration of each stage (in minutes) is shown in columns 2 and 3; the percentage of the sleep episode (8 h for BL, 6.7 h for FD) is shown in columns 4 and 5. NREM refers to non-rapid eye movement sleep; REM, rapid eye movement sleep; SWS, slow wave sleep (stage 3 and 4); Wake, wakefulness during scheduled sleep (% of time in bed after lights out); SE, sleep efficiency (total sleep time/time in bed after lights out x 100); SL, sleep latency in min; SOFF, sleep offset (wakefulness before lights on; min). Parentheses indicate F values.

*Stages in which 2-way repeated analysis of variance showed a significant main effect (P < 0.05) of condition between BL and FD, or a main effect of sex.
When we compared FD sleep that occurred during the biological night with that from the biological day, we found that, during the biological night, subjects slept significantly longer, had higher SE, and more stage 2 sleep, and less wakefulness and stage 1 sleep (Table 2). During the biological day, SL was significantly shorter, and sleep offset occurred earlier within the scheduled sleep episode. During both conditions, compared with men, women slept significantly longer overall and had less wakefulness during the scheduled sleep episodes, and, thus, their SE was higher during both conditions (main effect of sex; *P* < 0.05).

### EEG Sleep Spectra During NREM Sleep

#### BL vs FD

Across the FD sleep episodes, EEG activity was significantly higher in the SWA (0.5 to 5.25 Hz), theta (6 to 6.25 Hz; 7 Hz), alpha (10 to 11.25 Hz), and higher spindle (14.5 to 15.5 Hz) ranges than during the BL (3-way rANOVA on absolute data with the factors “order,” “sex,” “condition” [BL, FD]; main effect of condition; *P* < 0.05; see Figure 4). During the BL and FD, women had significantly higher EEG activity in the SWA, theta (0.5 to 1.75 Hz, 3 to 5.5 Hz), and lower spindle (13.5 to 13.75 Hz) ranges than men (main effect of sex; *P* < 0.05). There was no main effect of order or a significant interaction of the 3 factors. A potential order effect was also tested by performing a 1-way analysis of variance on relative data (FD as a percentage of BL) and revealed a main effect of order in only 2 narrow EEG frequency bins (between 3.25 to 3.5 Hz and at 17.5 Hz), with higher values for those subjects who had the placebo condition first (*P* < 0.05).

### Separation of Circadian and Homeostatic Effects

To investigate the circadian and homeostatic influences on NREM sleep, we analyzed the EEG spectra from the 8 circadian phase bins and the 4 homeostatic sleep-dependent intervals (see Methods) across the frequency range between 0.5 and 25 Hz. A mixed-model analysis with the fixed effects “circadian phase” and “homeostatic interval” was done for each 0.25-Hz bin and revealed a significant circadian modulation in the EEG delta to 13.25 Hz), high spindle frequency (Hsfa; 14 to 15 Hz) and lower spindle (13.5 to 13.75 Hz) ranges than men (main effect of sex; *P* < 0.05). There was no main effect of order or a significant interaction of the 3 factors. A potential order effect was also tested by performing a 1-way analysis of variance on relative data (FD as a percentage of BL) and revealed a main effect of order in only 2 narrow EEG frequency bins (between 3.25 to 3.5 Hz and at 17.5 Hz), with higher values for those subjects who had the placebo condition first (*P* < 0.05).

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### Table 2—Sleep stages during biological night and biological day in forced-desynchrony

| Sleep stage, % of TST | Biological night sleep | Biological day sleep | Night-day (F<sub>1,23</sub>) | Sex (F<sub>1,23</sub>) |
|-----------------------|------------------------|----------------------|-------------------------------|------------------------|
| NREM                  | 66.2 ± 5.3             | 64.4 ± 6.3           | 1 (9.5)                       | 5 (5.0)                |
| REM                   | 24.3 ± 4.3             | 22.4 ± 7.7           | 1 (4.7)                       | 5 (5.0)                |
| SWS                   | 16.8 ± 8.9             | 19.9 ± 7.8           | 1 (23.7)                      | 5 (5.0)                |
| 1                     | 9.5 ± 5.2              | 13.1 ± 5.9           | 1 (9.5)                       | 5 (5.0)                |
| 2                     | 49.4 ± 9.1             | 44.6 ± 9.5           | 1 (23.7)                      | 5 (5.0)                |
| Wake, %               | 15.7 ± 10.7            | 34.6 ± 14.3          | 1 (23.7)                      | 5 (5.0)                |
| TST, min              | 336.8 ± 42.9           | 260.5 ± 56.7         | 1 (24.0)                      | 5 (5.0)                |
| SE, %                 | 84.3 ± 10.8            | 65.1 ± 14.1          | 1 (24)                        | 5 (3)                  |
| SL, min               | 7.2 ± 6.2              | 4.6 ± 4.9            | 1 (7.5)                       | 5 (3)                  |
| SOFF, min             | 7.4 ± 30.1             | 43.0 ± 51.5          | 1 (17.0)                      | 5 (3)                  |

Notes: Sleep-stage characteristics (mean ± SD; N = 23) for 6.7-h forced-desynchrony (FD) sleep episodes occurring during the biological night and biological day.

NREM refers to non-rapid eye movement sleep; REM, rapid eye movement sleep; SWS, slow wave sleep (stage 3 and 4); Wake, wakefulness during scheduled sleep (% of time in bed after lights out); SE, sleep efficiency (total sleep time/time in bed after lights out x 100); SL, sleep latency (min); SOFF, sleep offset (wakefulness before lights on; min).

*Stages in which 2-way repeated analysis of variance showed a significant main effect (P < 0.05) of condition between biological night and biological day, or a main effect of sex; F values are indicated in parentheses.*
episode, whereas, in contrast, HSFA increased in the latter half of the sleep episode. EEG beta activity did not vary within the sleep episode. For the purpose of illustration, we binned the EEG sleep spectra into “classic” EEG frequency bins and then averaged them across subjects (see Figure 5), similar to what was reported by Dijk and colleagues from young subjects. The factors “circadian phase” and “homeostatic interval” showed a significant interaction in the EEG delta range (0.5 to 1.5 Hz). Posthoc analysis revealed significantly higher EEG delta activity during the first quarter of scheduled sleep when this segment of sleep occurred in the early afternoon (at the 135° bin) when compared with later circadian phases (P < 0.04; see Figure 6).

Biological Night-Day Differences
When comparing FD sleep episodes that occurred during biological night with those that occurred during biological day, we found significantly higher relative EEG power density during the biological day than the biological night in the EEG SWA, theta, and alpha ranges (0.5 to 12 Hz), as well as in the high spindle and beta ranges (14.25 to 21 Hz and at 21.5 Hz; P < 0.05; 2-way rANOVA; main effect of “biological time.”) Women had generally greater EEG activity in the high spindle range than men (14.75 to 16.25 Hz; main effect of “sex”; P < 0.05). Additionally, there was a significant interaction of the factors “biological time” and “sex” at 10.5 to 11.25 Hz and at 13 Hz, with women having significantly lower EEG activity during the biological day than men (Duncan multiple range test; P < 0.04; see Figure 7).

When compared with BL sleep, EEG spectra during the biological night of FD were higher in only parts of the SWA range (1 to 2.25 Hz), whereas EEG spectra during the biological day of FD were higher than BL in most frequency bins (0.5 to 11.75 Hz and 14.25 to 17.5 Hz; 1-way rANOVA; P < 0.05).

Correlation of EEG Sleep Stages and Spectra
To test whether subjects with less consolidated sleep also had a higher EEG beta activity, we correlated EEG beta activity (15.75 to 20.5 Hz) with TST and with wakefulness during scheduled sleep for each subject (see Methods). Overall, there was a significant negative correlation between EEG beta activity and TST (r = -0.48; P < 0.05) and a significant positive correlation between EEG beta activity and wakefulness (r = 0.53, P < 0.05; see Figure 8 and Table 3). There was also a significant negative correlation between TST and wakefulness (r = -0.76; P < 0.05). This indicates that those subjects who had less consolidated sleep had a higher variation in beta activity. These overall correlations across all circadian phases were significant for both sexes when we tested them separately (P < 0.05). When we compared the correlations at different circadian phases, EEG beta activity was significantly correlated with wakefulness in 5 of the circadian phase bins (0°, 45°, 135°, 180°, and 270°), whereas EEG beta activity showed a significant negative correlation with TST at only 2 of the circadian phase bins (135° and 180°; P < 0.05). TST and wakefulness were negatively correlated (r > -0.48; P < 0.05) in all but 2 circadian phase bins (during the biological night, at 315° and 360°).

We also correlated EEG delta activity (0.5 to 1.5 Hz) in the first (homeostatic) sleep interval with TST and wakefulness but did not find any significant overall correlations. (r = -0.05, P > 0.46 for wakefulness; r = 0.05, P > 0.46 for TST; see Table 3) nor did we find significant correlations in any circadian bin (P > 0.12).

DISCUSSION
There are prominent age-related changes in sleep consolidation and evidence of age-related changes in both of the major sleep regulatory processes—the circadian timing system and the homeostatic sleep-dependent influence on sleep. Work by our group and others suggests that it is the consolidation of NREM sleep that is most affected by aging, and, thus, we conducted the analyses presented here to examine in detail how the sleep EEG is affected by circadian phase, by time within sleep, and by their interactions. We examined EEG sleep spectra in healthy older subjects scheduled to sleep at many circadian phases in an FD study to examine how sleep at different times of day differs from baseline sleep, how circadian phase impacts the sleep EEG, and how circadian phase and time within sleep impact the EEG.
Figure 5—Circadian-dependent (left side; double-plotted) and homeostatic-dependent (right side) electroencephalographic (EEG) activity during forced desynchrony (FD). EEG spectra were averaged across multiple frequency ranges as indicated in the right label: slow-wave activity (SWA): 0.75-4.5 Hz; theta activity: 4.75-7.5 Hz; alpha activity: 8-12 Hz; low spindle frequency activity (LSFA): 12.25-13.75 Hz; high spindle frequency activity (HSFA): 14-15.5 Hz; beta activity: 15.75-24.75 Hz (N = 24; ± SEM). Dotted line at 0° in the left panel indicates the time of the core body temperature minimum.
Our quantification of EEG NREM spectra from FD sleep episodes revealed significantly higher EEG power density in the lower frequency (≤ 7 Hz), alpha, and high spindle ranges, compared with BL. When we examined sleep occurring at specific circadian phases, we found that, when sleep was scheduled in the morning or early afternoon, there was a significant interaction of circadian and homeostatic processes in the EEG delta range, presumably caused by higher sleep pressure at the beginning of the sleep episodes. Our comparison of sleep episodes that occurred during the biological night versus the biological day (when endogenous melatonin was present or absent) found a higher EEG SWA, alpha, theta, high spindle, and beta activity when sleep occurred during the biological day.

**EEG Sleep Stages During BL and FD**

The average sleep stage duration during BL nights in our older subjects was comparable with data from prior studies. Overall sleep opportunity per 24 hours in the FD protocol was, on average, 8.04 hours, compared with 8.0 hours on the BL nights, but the length of each scheduled sleep episode during the FD condition was shorter than during the BL nights, and, therefore, TST was shorter during the FD sleep episodes than during the BL. Despite equal sleep opportunities per 24 hours during the BL and the FD, the subjects could not take full advantage of the scheduled FD sleep episodes and, overall, showed significantly lower SE during the FD nights, which was most pronounced at the end of the scheduled sleep episodes. There was a similar duration of SWS during FD and BL, but, because of the shorter duration of each scheduled sleep episode during the FD (when compared with BL), this resulted in a relatively higher percentage of SWS during the FD. Compared with a prior study in young subjects with the same imposed sleep-

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**Figure 6**—Interaction of circadian and homeostatic effects in the electroencephalographic (EEG) delta range. EEG delta power (0.5-1.5 Hz) was averaged with respect to circadian phase for each quarter of the scheduled sleep episode and is shown double-plotted. N = 24; ± SEM.

**Figure 7**—Averaged electroencephalographic (EEG) spectra relative to baseline (100% = first 6.7 hours of baseline) during sleep episodes scheduled in the biological night (upper panel) and biological day (lower panel) for women (filled circles) and men (open circles). Black upward triangles indicate frequency bins in which there are significant sex differences, white triangles indicate frequency bins in which there are significant differences between biological day and night, and black downward triangles indicate frequency bins in which there are significant interactions between sex and day/night condition. N = 23 (13 F, 10 M); ± SEM.
Higher EEG SWA During FD than BL

In our comparison of EEG sleep spectra between FD and BL, we used only the first 6.7 hours of scheduled BL sleep so as to have the same durations. Our finding of higher EEG activity in the SWA range during the FD when compared with the BL is therefore unlikely to be due to the different lengths of the data included, and also unlikely to be caused by the different lengths of the scheduled sleep episodes. Instead, we assume that the difference in SWA was due to the imposed 20-hour sleep-wake schedule in the FD protocol, which resulted in circadian misalignment, leading to overall reduced SE and, in turn, higher sleep pressure. In fact, there was significantly greater EEG SWA during biological day sleep and, to a smaller extent, during night sleep, when compared with BL. In contrast, SE was high during the biological night and very low during biological day, compared with BL. One explanation for this finding is that these older subjects slept more “intensely” during biological daytime. This idea is supported by 2 lines of evidence. First, under normal nighttime sleeping conditions, most SWA occurs in the first half of a sleep episode, with a progressive decline across the night. And, thus, sleep debt (as indexed by SWA) is neutralized mostly at the beginning of the sleep episode. Moreover, it has been shown previously in young adults that more SWA occurs during daytime sleep, when REM sleep propensity is lowest. Our present results are consistent with this latter finding and earlier reports, suggesting that the distribution of NREM-REM sleep across the circadian cycle is preserved with age. In addition, we believe that the reported age-related narrowing of circadian phases at which consolidated sleep can be maintained could have contributed to the current finding of higher EEG SWA during daytime sleep. During the FD, when wakefulness is scheduled at adverse circadian times (e.g., during the biological night), sleep pressure builds up during wakefulness but cannot be completely dissipated during the following daytime sleep episode because of the difficulty older subjects have maintaining sleep at adverse circadian phases. Because of this inability to take advantage of the scheduled sleep opportunities, it is likely that the subjects did not get as much sleep during the FD as they needed. Taken together, presumably both homeostatic and circadian sleep-regulatory mechanisms led to day-night differences in sleep, contributing to the overall increase of EEG SWA during FD, when compared with baseline levels.

Circadian- and Homeostatic-dependent Effects on Sleep Stages and EEG Delta Activity

The homeostatic aspect of this finding described above is presumably due to the shorter duration of FD sleep episodes and a higher accumulated sleep pressure. The sleep stage data support this, indicating that these older subjects could not maintain a consolidated sleep episode during the FD, showing a significant decrease of TST and an increase of wakefulness in the second half of their 6.7-hour scheduled sleep episodes. The circadian aspect of the interaction in the EEG delta range could be explained by its time course across circadian phase. We found that EEG delta power during the first quarter of the scheduled

Table 3—Correlation coefficients for wakefulness, TST, and EEG beta and delta activity

| Correlation variables | r (t) |
|-----------------------|------|
| Wake X EEG Beta       | 0.53* (8.55) |
| TST X EEG Beta        | -0.48* (-7.53) |
| TST X Wakefulness     | -0.76* (-16.2) |
| Wake X EEG Delta      | -0.05 (-0.74) |
| TST X EEG Delta       | 0.05 (0.74) |

Notes: Correlation between wakefulness, total sleep time (TST), and electroencephalographic (EEG) beta activity (15.75-20.5 Hz) and EEG delta activity (0.5-1.5 Hz; in the first homeostatic sleep interval) across circadian phases. Correlation coefficient (r) and its corresponding t value are presented. N = 24.

*Significant correlations at P < 0.05.
sleep episodes was higher when sleep occurred in the morning and early afternoon than when sleep occurred during other circadian phases. Those sleep episodes would have occurred on the FD cycle following sleep episodes scheduled in the early afternoon and evening. We found that early afternoon-evening sleep episodes were less consolidated, especially in the second half, as indexed by less TST and more wakefulness. Thus, sleep loss in an afternoon-evening sleep episode was, in general, followed by greater delta activity in the following morning-afternoon sleep episode. In general, it is not well understood how the circadian system regulates sleep during the daytime because, in humans, the major sleep episode occurs during the night. A limitation of our results is that we cannot determine whether our finding of greater delta activity in sleep episodes scheduled in the morning and early afternoon represents an age-related effect of circadian regulation of daytime sleepiness, an effect of greater homeostatic sleep pressure due to the preceding sleep and wake episodes, or an effect of cumulative sleep restriction as sleep was lost across the FD.

Circadian- and Homeostatic-dependent Effects on EEG Spindle Activity

We observed a circadian modulation in the high, but not the low, EEG spindle range across the FD sleep episodes in these healthy older subjects. In the lower EEG spindle range, we found a circadian modulation in only two 0.25-Hz EEG frequency bins (13 to 13.25 Hz), confirming the previously reported age-related attenuation in this EEG range, as well as the previously reported attenuation of incidence, frequency, and amplitude of sleep spindles in older adults.

In young adults, there is a strong correlation between sleep pressure and EEG spindle frequency, such that increasing sleep pressure reduces EEG spindle frequency. In a prior study in young adults, Dijk and colleagues reported that, as sleep progressed, there was an increase in EEG activity in the entire EEG spindle range. In the older subjects in the present study, we found an increase in the high spindle frequency activity as sleep progressed but no such change in the low spindle frequency range. Our findings confirm an age-related reduction of the circadian variation in the lower spindle range, together with an attenuated homeostatic influence in the lower spindle range (as the sleep episode progressed). Low-frequency EEG sleep spindles have been hypothesized to serve a sleep-protecting function, and the attenuated amplitude of low-frequency spindles in older subjects could have contributed to the sleep-maintenance difficulty our subjects had when sleeping during the biological daytime.

Circadian- and Homeostatic-dependent Effects on EEG Beta Activity

We found a circadian modulation in the EEG beta range in the older subjects in our study, with a peak during the daytime and a nadir during the late biological night. Moreover, we found that the EEG beta activity of our subjects was positively correlated with wakefulness and negatively correlated with TST. Our findings are in accordance with those of a multiple-nap study, in which older subjects showed a circadian rhythm of EEG beta activity. This is in contrast to our prior report from a 28-hour FD study in young adults, in which we found no circadian modulation of EEG beta activity. Thus, the occurrence of increased EEG activity in the higher frequency ranges could be age dependent, as has been hypothesized previously.

The EEG beta rhythm is understood to be an index of physiologic arousal and cognitive functioning in humans and during sleep is increased in patients with primary insomnia. In fact, a positron emission tomography study revealed an increase of relative cerebral blood flow in the ascending arousal system and in cortical areas in insomniacs compared with good sleepers. In healthy individuals, EEG beta activity during night sleep has been shown to be increased in middle-aged subjects when compared with young subjects, mostly during the first NREM sleep cycle. The authors of that report hypothesized that the increase of EEG beta activity across the night in middle-aged subjects led to increased cortical activity during sleep, making them more vulnerable to sleep disruption. Therefore, the relative increase in EEG beta activity in our older subjects during daytime sleep and its differential correlation with TST and wakefulness could reflect a higher cortical activity, which, if present, could have contributed to their inability to maintain sleep.

Sex

We found an influence of sex, such that the women in our study had significantly greater amounts of sleep and less wakefulness during scheduled sleep than did the men during both BL and FD. The women also had higher EEG SWA and lower spindle activity in both BL and FD conditions, similar to the results published in prior reports, although a causal explanation for these sex differences remains to be elucidated. In 1 of those prior reports, it was hypothesized that differences in EEG potential strength due to sex variation in skull thickness could be a contributing factor. We also found time-of-day-dependent differences between men and women, with men having higher EEG alpha and higher EEG spindle activity (at 13 Hz) during the biological day, which suggests there could be sex-specific differences in circadian sleep regulation. However, the men in our study were slightly older than the women, so some of the sex differences we observed could be due in part to the age differences between the sexes.

CONCLUSION

Our findings show the contribution of homeostatic and circadian processes to the EEG sleep stages and spectra of older subjects during NREM sleep when sleep was scheduled over many circadian phases. Our findings provide some evidence for age-related altered homeostatic and circadian sleep-wake regulation. In addition, the negative correlation between TST and EEG beta activity during daytime sleep provides some new evidence for the underlying mechanisms that contribute to age-related difficulties in sleep consolidation, especially when sleep occurs at adverse circadian times.

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