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Original Article

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Napping and circadian sleep–wake regulation during healthy aging

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Abstract

Study Objectives: Daytime napping is frequently reported among the older population and has attracted increasing attention due to its association with multiple health conditions. Here, we tested whether napping in the aged is associated with altered circadian regulation of sleep, sleepiness, and vigilance performance.

Methods: Sixty healthy older individuals (mean age: 69 years, 39 women) were recruited with respect to their napping habits (30 nappers, 30 non-nappers). All participants underwent an in-lab 40-hour multiple nap protocol (10 cycles of 80 minutes of sleep opportunity alternating with 160 minutes of wakefulness), preceded and followed by a baseline and recovery sleep period. Saliva samples for melatonin assessment, sleepiness, and vigilance performance were collected during wakefulness and electrophysiological data were recorded to derive sleep parameters during scheduled sleep opportunities.

Results: The circadian amplitude of melatonin secretion was reduced in nappers, compared to non-nappers. Furthermore, nappers were characterized by higher sleep efficiencies and REM sleep proportion during day- compared to nighttime naps. The nap group also presented altered modulation in sleepiness and vigilance performance at specific circadian phases.

Discussion: Our data indicate that napping is associated with an altered circadian sleep–wake propensity rhythm. They thereby contribute to the understanding of the biological correlates underlying napping and/or sleep–wake cycle fragmentation during healthy aging. Altered circadian sleep–wake promotion can lead to a less distinct allocation of sleep into nighttime and/or a reduced wakefulness drive during the day, thereby potentially triggering the need to sleep at adverse circadian phase.

Key words: aging; napping; circadian sleep–wake regulation; REM sleep

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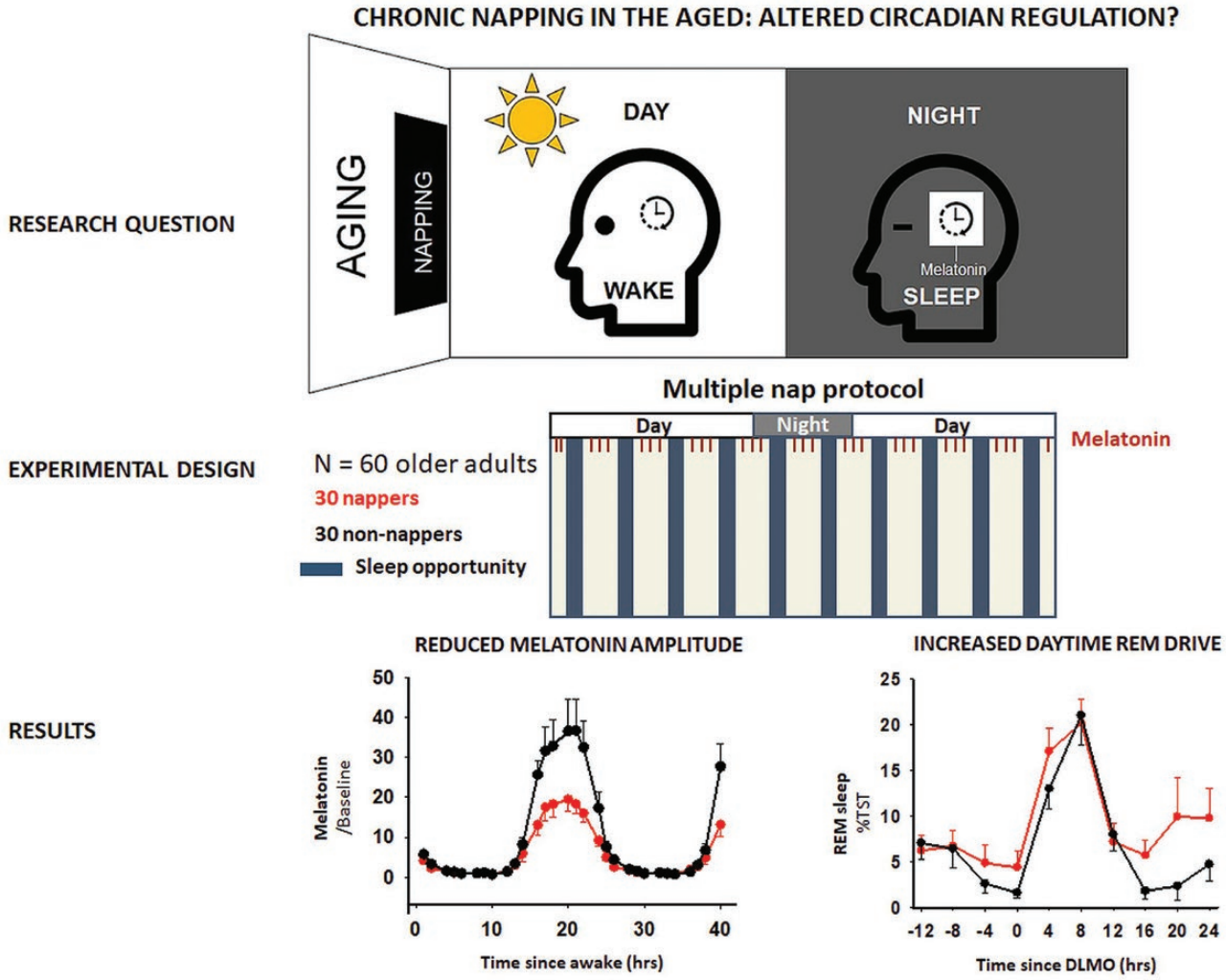
Graphical Abstract



Significance of Statement

Although napping has raised increasing interest as a health risk factor in epidemiological studies, its underlying regulation processes in the aged remain largely elusive. Here we assessed whether napping in the older population is associated with physiological and behavioral changes in circadian sleep–wake characteristics. Our data indicate that, concomitant to a reduced circadian amplitude in melatonin secretion, healthy older nappers are characterized by reduced day–night differences in sleep efficiency and more particularly in REM sleep, compared to their non-napping counterparts. These results suggest altered circadian response as a cause or consequence of chronic napping in the aged and thereby contribute to the understanding of nap regulation during healthy aging.

Introduction

The circadian clock provides the gross temporal framework for sleep and wakefulness alternation over the 24-hour cycle. Under entrained conditions, the human circadian system is timed to achieve consolidated periods of sleep during nighttime and a continuous period of wakefulness during the day, by acting through adaptive arousal mechanisms that oppose the wake-dependent built-up of homeostatic sleep pressure [1]. Maximal circadian wake promotion occurs towards the end of a classical waking day to maintain wakefulness despite increasing sleep pressure levels. In contrast, maximal circadian sleep promotion occurs towards the end of the biological night, to maintain consolidated sleep despite dissipating sleep pressure levels. The fine-tuned interaction between circadian and sleep homeostatic processes thus favors a monophasic distribution of sleep and wakefulness over the 24-hour light–dark cycle, which is considered a milestone in human ontogenesis [2]. In contrast, the perturbation of the interaction between these processes leads to fragmentation of sleep and wakefulness states and associated deterioration in neurobehavioral performance [1, 3].

Alterations in components of the circadian and sleep homeostatic systems have been suggested to contribute to age-related modifications in sleep habits [4–6]. With respect to the circadian system, older adults generally present an advanced phase of circadian rhythmicity in classical endocrine markers (e.g. melatonin and cortisol [7]). The amplitude of circadian sleep and/or wake

propensity levels has also been suggested to be affected by aging [8–10]. As mentioned previously, a perturbation of the interaction between sleep homeostatic and circadian processes predicts fragmented sleep–wake cycles, including reduced monophasic sleep distribution. As such, chronic napping may reflect a visible manifestation of underlying altered sleep regulation processes and even more particularly altered circadian sleep propensity levels. Assessing this hypothesis is relevant considering that chronic napping habits increase with advancing age such that more than half of adults aged 70 and above nap at least twice a week [11, 12].

Besides cultural differences and rather independent of age, people classically nap in response or prior to sleep loss, as a countermeasure for daytime sleepiness, stress relief, or simply because of nap enjoyment. Napping acutely reduces self-reported sleepiness and has the potential to improve well-being or cognitive performance [11], including beneficial effects for sleep-dependent memory consolidation [13]. Similarly, splitting sleep with a mid-afternoon nap offers a boost to neurobehavioral performance [14–16] and vigilance-related time-on-task decrement [17] in adolescents, the effects of which however differ according to total sleep opportunity over the 24-hour cycle [16].

While the beneficial effects of napping, used for example as an acute countermeasure for sleep loss, have been extensively discussed, epidemiological studies increasingly point towards chronic napping as a health risk factor in the aged, not only for medical comorbidities and increased mortality [18–20], beta-amyloid burden [21, 22], but also for age-related cognitive decline [23–25]. More recently, frequent- and long-duration daytime rests have been suggested to predict the incidence of Alzheimer's disease [26]. This may be particularly true if napping is perceived as a need, starts to occur frequently, with a long duration, and occurs unintentionally in the context of the aging brain [27]. Despite the increasing interest in napping from an epidemiological perspective, the regulation processes underlying napping in the aged remain largely elusive. A critical feature of sleep and wakefulness that deteriorates with age is the ability to maintain these states over extended periods of time [28], such that older individuals may encounter difficulties staying asleep at night (sleep is fragmented) and maintaining waking alertness through the day (naps are more prevalent). In line, we recently observed that increased actimetry-derived daytime rest frequency is not only associated with an altered 24-hour rest-activity distribution, but also reduced episodic memory performance [29]. We also observed that individuals who rest later in the day go to bed later with respect to their circadian phase, thereby indicating circadian misalignment.

The aim of the current study was to assess whether napping in older adults is associated with physiological and behavioral changes in circadian modulation. To do so, a matched group of healthy older nappers and non-nappers underwent a 40-hour multiple nap protocol under controlled laboratory conditions. Circadian rhythm amplitude in melatonin secretion, as well as 24-hour modulations in sleep parameters and waking performance, were compared between groups. We expected that nappers are characterized by reduced circadian amplitude, translated into a less distinct allocation of sleep ability during night- compared to day-time and/or a reduced modulation of vigilance and sleepiness levels over the 24-hour cycle.

Methods

This cross-sectional study was part of a larger research project. Data considered here were collected during three phases: (1) a telephone interview and screening visit, (2) a pre-laboratory field actimetry study, and (3) an in-laboratory study, encompassing a 56-hour stay. Melatonin and actimetry data from a sub-sample of individuals included here have been published previously [29].

Participants

Recruitment aimed at covering a wide range of socioeconomic classes and was performed via advertisement in newspapers, radio and university and by taking advantage of already existing GDPR-compliant databases at the research unit. Volunteers were retired and lived at home. Seven hundred and seventy three individuals aged > 60 were initially contacted. Out of this sample, 94 individuals were retained after an eligibility check and a screening night of polysomnography (see also below). None of the participants indicated moderate or severe depression (Beck Depression Inventory [30] [BDI-II] < 19) or severe anxiety (Beck Anxiety Inventory [31] [BAI] < 30). Clinical symptoms of cognitive impairment were assessed by the Mini-Mental State Examination [32] (MMSE score > 26) and the Mattis Dementia Rating scale [33] (MDR score > 130). Screening for major sleep disorders was performed during a night of polysomnography (apnea–hypopnea index: 5.48 ± 4.63 (mean ± SD), periodic limb movement index: 3.07 ± 5.51 (mean ± SD). Other exclusion criteria included body mass index (BMI) ≤ 18 and ≥ 30 kg/m^2, participants reporting a history of diagnosed psychiatric conditions or severe brain trauma, chronic medication affecting the central nervous system (e.g. sleep medication, anxiolytics, beta blockers), diabetes, smoking, caffeine (>4 cups/day), excessive alcohol (>14 units/week) or other drug consumption, and traveling more than one-time zone in the 3 months prior to the study begin. Participants with stable treatment (>6 months) for hypertension and/or hypothyroidism were included in the study.

For this cross-sectional assessment, a group of 30 nappers and 30 non-nappers were selected out of the sample and matched at the group level with respect to age, gender, educational level, and season of assessment. Demographic characteristics of the groups are summarized in Table 1. To be part of the nap group, individuals had to regularly nap at least twice a week, for at least 30 minutes and for at least 1 year, as assessed by a questionnaire. The non-napping group consisted of individuals declaring not or to only occasionally nap and/or not reaching the nap criteria to be part of the nap group. Daytime rest duration and frequency were retrospectively assessed through actimetry recordings in both groups (see also below). Sample size estimation (n = 30 per group) was based on previous literature reports on age-related effects in the circadian regulation of sleep and behavioral outcomes similar to those assessed here [9].

Study procedure

The study procedure is depicted in Figure 1. After study enrollment, participants underwent a night of polysomnography in the lab, to screen for apnea–hypopnea and periodic limb movements. Then, participants wore an actigraph and completed a sleep diary for at least 8 days while maintaining daily routines and self-selected sleep schedules at home in order to assess daytime rest habits. This recording was used to confirm the individual's nap phenotype according to the predefined criteria. Once confirmed, the constant routine, including a week of fixed actimetry preceding the in-lab session, was scheduled. During this week, participants were asked to keep a fixed sleep–wake schedule for 7 days to ensure sufficient sleep at night (8 hours ± 30 minutes time

Table 1. Demographic, Questionnaire and Actimetry Data (Means, Standard Deviations) by Group

Sample characteristics	NAP	NON-NAP	*p*
N (f,m)	30 (21,9)	30 (18,12)	
Age (y)	69.2 ± 5.25	69.37 ± 5.89	
BMI (kg/m^2)	25.84 ± 2.83	24.41 ± 2.50	**0.04**
BDI	3.93 ± 3.03	3.2 ± 3.19	
BAI	3.23 ± 3.72	2.77 ± 3.52	
PSQI score	5.03 ± 2.66	4.93 ± 2.91	
ESS score	9.07 ± 3.18	6.50(3.42)	**0.006**
MEQ score	63.17 ± 8.44	63.13 ± 7.40	
SPAQ score	8.17 ± 4.71	4.97 ± 4.49	**0.005**
Wake time (hh:mm) during study	07:14 ± 00:45	07:14 ± 00:33	
MMSE (total)	29.60 ± 0.62	29.52 ± 0.69	
PACC-5	−0.10 ± 2.54	0.12 ± 2.99	
Educational level (y)	14.60 ± 2.91	14.13 ± 3.58	
DMLOn (hh:mm)	21:40 ± 1:25	21:23 ± 1:08	
Phase angle (min)	54.73 ± 95.48	77.13 ± 66.42	
DTR daily frequency	0.73 ± 0.32	0.26 ± 0.21	**0.006**$^{-0}$
DTR duration (min)	49.95 ± 14.22	32.87 ± 21.79	**0.0007**
DTR timing (distance from DLMOn)	−419.10 ± 93.63	−411.73 ± 112.88	

f, female; m, male; y, years; BMI, body mass index; BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory; PSQI: Pittsburgh Sleep Quality Index; ESS, Epworth Sleepiness Scale; MEQ, Morningness–Eveningness Questionnaire; SPAQ, seasonal pattern assessment questionnaire; MMSE, mini-mental state examination; PACC-5 score, Preclinical Alzheimer's Composite Score; DLMOn, dim light melatonin onset; DTR, Daytime rest as extracted from actimetry. Only significant differences are reported, i.e. $p > 0.05$ if no p-value is provided.

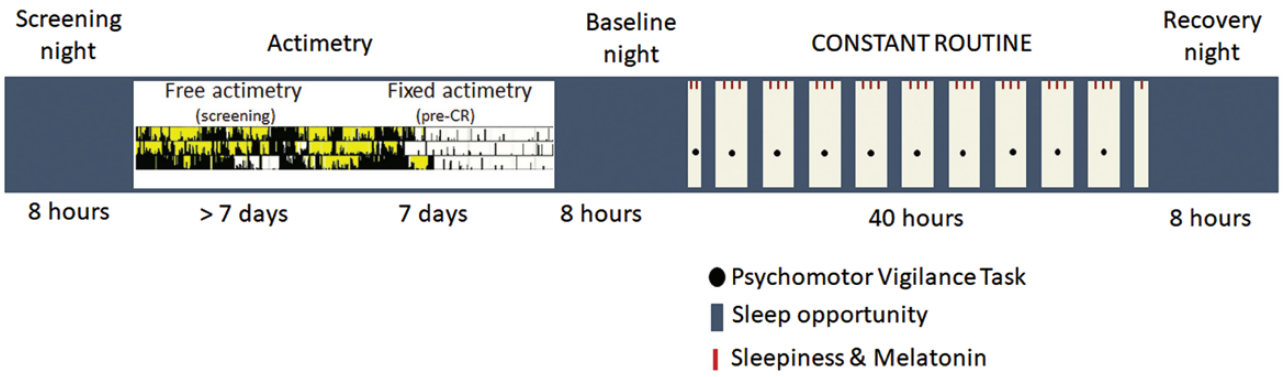


Figure 1. Schematic representation of the study protocol including a screening night of polysomnography, followed by daytime rest characterization in the field using actimetry and concomitant assessment of light exposure (>7days for screening actimetry, 7 days for fixed actimetry with the instruction to keep a regular and predetermined schedule before laboratory entrance). The in-lab protocol started with an 8-hour baseline night (BAS), monitored by polysomnography. Thereafter, participants underwent a 40-hour multiple nap constant routine protocol, encompassing 10 short sleep–wake cycles, each consisting of 160 minutes of wakefulness alternating with 80 minutes of sleep opportunities (black). The protocol was followed by an 8-hour sleep opportunity (REC, recovery night). Light levels (<5 lux during wakefulness and 0 lux during sleep), temperature (~19°C), caloric intake and body posture (semi-recumbent position during scheduled wakefulness and recumbent during naps) were controlled. Salivary melatonin and self-reported sleepiness (short lines) were regularly collected through the 40-hour. Polysomnography was recorded during scheduled sleep opportunities (rectangles). Psychomotor vigilance performance was assessed after each nap opportunity (dots).

in bed) and stable circadian entrainment. Sleep schedules were individually adapted to the participants' habitual sleep–wake times, centered on an 8-hour sleep opportunity. Participants were allowed to maintain their daily routines with respect to napping habits. Thus, no specific recommendations or restrictions about napping were provided during the actimetry recordings to maintain the participant's corresponding nap phenotype. Adherence to the schedule was verified by visual inspection of actigraphic recordings. If daylight saving time occurred before the constant routine protocol, participants (two in our sample) were asked to keep the time before the change for an extra-day. Participants were instructed to abstain from alcohol and caffeine during this week to prevent withdrawal effects. After the baseline night at the laboratory, participants underwent a 40-hour multiple nap protocol encompassing 10 short sleep–wake cycles of 80 minutes of sleep opportunity (i.e. a nap) alternating with 160 minutes of wakefulness. The first cycle started 130 minutes after scheduled wake-up time from the baseline night. The duration of wakefulness in the last cycle was restricted to 40 minutes such that the recovery night started at habitual sleep time. The experiment

was conducted under controlled conditions according to light input (<5 lux during scheduled wakefulness; 0 lux during scheduled sleep opportunities), isocaloric food intake (standardized meals every 4 hours), temperature (~ 19°C) and body posture (semi-recumbent position during scheduled wakefulness and recumbent during sleep opportunities; see also [29] for further specifications on light settings). Participants were not allowed to stand up, except for regularly scheduled bathroom visits and did not have any indications of time of day. Social interaction was restricted to communications with study helpers. Salivary melatonin was collected at regular intervals (~1.25 hours) throughout the 40-hour protocol. During scheduled wakefulness, participants had to complete self-reported sleepiness scales and mood ratings every hour and to perform a 10-minute psychomotor vigilance task 1 hour after lights-on of each scheduled nap opportunity. Polysomnography recordings were performed during sleep opportunities over the protocol. Note that data acquired during the first day may be masked by confounds, including the habituation to the imposed sleep–wake regime, inherent group differences in the ability to get habituated to such regime, potential interindividual differences in nighttime sleep parameters or unmet sleep need despite the 8-hour baseline sleep opportunity, which may be washed out during the first naps of the protocol. Accordingly, even though, for the sake of completeness, the statistical model includes all acquisition sessions (see also below), the discussion of the sleep and vigilance variables of the current paper put the focus on the comparison between night acquisitions and those performed during the second day, which should more accurately reflect the individual's circadian sleep–wake-promoting drive.

The study was approved by the local Ethics Committee of the University Hospital and of the Faculty of Psychology, Speech Therapy, and Educational Sciences at the University of Liège (Belgium) and performed in accordance with the Declaration of Helsinki. Participants gave written informed consent and received financial compensation.

Questionnaires and cognitive status

In addition to questionnaires used for eligibility checks, the Pittsburgh Sleep Quality Index [34] (PSQI), the Epworth Sleepiness Scale [35] (ESS), the Morningness–Eveningness Questionnaire [36] (MEQ), as well as the Seasonal Pattern Assessment Questionnaire [37] (SPAQ) were administered at study entrance. Overall cognitive status was further assessed by computing the Preclinical Alzheimer's Disease Composite Score (PACC-5 [38]). The PACC-5 reflects a composite measure of a series of cognitive tests, usually used in clinical settings and encompassing episodic memory, timed executive function, and global cognition. The PACC-5 is calculated as mean performance across 5 measures including the MMSE (0–30), the WMS-R Logical Memory Delayed Recall (LMDR [39]; 0–25), the Digit-Symbol Coding Test (DSC [40]; 0–93), the Free and Cued Selective Reminding Test–Free + Total Recall (FCSRT [41]; 0–96) and performance on a verbal fluency task (categorial fluency, 1-minute trials for generation of items belonging in the categories of animals, fruits, and vegetables).

Daytime rest assessment: actimetry

Participants wore an actigraph (Motionwatch 8, CamNtech, UK) at the non-dominant wrist and completed a sleep diary for at least 8 consecutive days, with a maximum duration of 15 days (13.6 ± 1.9 days [mean ± SD]). Locomotor activity was extracted from actigraphs with the Motionware software; reconstructed on the 3-axes, aggregated into 30-second epochs and processed by the open-source software pyActigraphy (v1.0; [42]). Periods of actigraph removal were visually identified according to sleep diaries and excluded from the analysis. For the actimetry screening session, the start time of the recording was delayed by 1 week if the daylight saving time occurred at the beginning of data collection (for five participants) or the recording was stopped at the time change if it was close to the end of data collection (for one participant).

The automatic scoring of the Munich Actimetry Sleep Detection Algorithm (MASDA [43, 44]) was used to detect consolidated rest periods over daytime with the following settings: at least 15 minutes with activity counts below 15% of the 24-hour centered moving average (see Supplemental Material for further specifications of the algorithm; see also [29] for sensitivity and specificity analyses when comparing the output using these settings to visual scoring of actimetry-derived rest periods in a cohort with similar demographic characteristics). We also compared actimetry-derived DTR characteristics scored by the MASDA algorithm and self-reported napping as derived from the sleep diaries of our participants. A rather sound correlation was observed between the number of automatically detected rest bouts by the MASDA and number of naps reported in the sleep diary (Kendall's rank correlation: $\tau = 0.48$, $p < 0.0001$). The same applied for duration (Kendall's rank correlation: $\tau = 0.30$, $p < 0.0001$) and timing (Pearson's correlation: $R = 0.80$, $p < 0.0001$).

Daytime was defined as the time window between the group-averaged dim light melatonin offset (DLMOff) + 2 hours and dim light melatonin onset (DLMOn) -2 hours, respectively. This time window was chosen to exclude potential confounding effects of transition periods during the early morning and late evening hours.

Three characteristics were extracted from actigraphy-derived daytime rest (DTR) bouts: (1) daily frequency, calculated as the mean number of DTR bouts per day, (2) duration, defined as the overall mean duration of DTR bouts, and (3) timing, defined as the median delay between DTR bouts start time and DLMOn. The latter could only be extracted when at least one rest bout was detected over the recording period ($n = 30$ nappers and $n = 25$ non-nappers).

Circadian phase and amplitude assessment: melatonin.

Saliva samples were obtained by passive drooling. No food intake was allowed 30 minutes prior to saliva samples and participants were not allowed any water intake and posture change for 15 minutes prior to collection. Salivary melatonin levels were analyzed via liquid chromatography coupled to a tandem mass spectrometer [45]. Secretion profiles were determined by fitting a skewed baseline cosine function to raw values [46]. Circadian phase was assessed by extracting the timing of DLMOn. The latter was defined as the point in time at which melatonin levels reached 25% of the fitted peak-to-baseline amplitude of individual data. Phase angles were computed by the distance between DLMOn and sleep time during the baseline night. Circadian amplitude was defined as the height of the fitted waveform with respect to its baseline.

Sleep electroencephalographic data acquisition and analysis

Seven electroencephalographic (EEG) channels (Fz, C3, Cz, C4, Pz, Oz, and O2), as well as two bipolar electrooculograms, and two

bipolar submental electromyograms, were used to assess sleep over baseline, nap, and recovery sleep opportunities. Signals were recorded using N7000 amplifiers (EMBLA, Natus Medical Incorporated, Planegg, Germany) with Ag/AgCl ring electrodes. The sampling rate was set at 500 Hz and signals were filtered online by applying a notch filter (50Hz). Sleep stages were automatically scored in 30-second epochs according to the American Academy of Sleep Medicine criteria AASM [47] using the ASEEGA sleep scoring algorithm (ASEEGA, PHYSIP, Paris, France). The algorithm has been previously used to score nighttime sleep in healthy young [48] and older [49] adults, but also in a series of sleep pathologies [50] and during daytime naps [51]. During each sleep opportunity, classical sleep parameters were extracted, including sleep efficiency (SE: sum of sleep stages 1, 2, 3, and REM divided by total sleep opportunity), sleep stage 1–3 (N1%, N2%, and N3%), as well as REM (REM%), expressed as a percentage over total sleep time (TST). Sleep latency to N1 and REM sleep and wake after sleep onset was also assessed during the baseline night.

Self-evaluation of sleepiness

Self-reported sleepiness was assessed by the Karolinska Sleepiness Scale [52] (KSS). Ratings were carried out at regular intervals (31 times over the 40-hour protocol, see Figure 1; three sessions per scheduled wakefulness between naps). Values were collapsed into 11 time bins (pooled per scheduled wake episodes between nap opportunities), by excluding the first assessment after each nap opportunity due to potential effects of sleep inertia.

Psychomotor vigilance performance

Vigilant attention performance was assessed using a modified version of the psychomotor vigilance task [53] (PVT) in 4-hour intervals at 10 time points over the protocol. To avoid sleep inertia effects on task performance, test timing was scheduled 1 hour after lights on from nap opportunities [54]. In this task, a white fixation cross was presented on a black computer screen. At random intervals (2–10 seconds), a millisecond counter started, and participants were instructed to press a button to stop the counter as fast as possible. Feedback of their reaction time (RT) performance was displayed for 1 second after their response. Duration of the task was set to 10 minutes. Lapse probability (defined as the number of trials with a RT > 500 ms, including time-out, divided by the total number of trials) was used as variable of interest, as it has been previously reported as sensitive to a state- and trait-like manipulation of sleep pressure levels and circadian phase [55, 56]. Follow-up analyses were performed on other classically derived metrics, including the mean of the 10% of fastest and slowest, as well as median RTs.

Statistics

Generalized linear mixed models (package glmmTMB; Brooks et al., 2017) were conducted using the statistics software R (R Core Team, 2020) to assess the effect of group (nap vs. non-nap), session (distance to DLMOn over the multiple nap protocol, 10 sessions of nap sleep opportunity, vigilance performance, as well as for self-reported scales) as well their interaction (group × session). Statistics were performed on data aligned to the individual's DLMOn and interpolated (third order bi-spline) values at the theoretical circadian phase of the protocol: –12, –8, –4, 0, 4, 8, 12, 16, 20, and 24 hours from DLMOn. Group and session were defined as categorical fixed effects and participants as random effect. Treatment contrasts were computed with the non-nap group as reference. For the session, the mean over the 2 night sessions (time since DLMOn + 4 hours, +8 hours) was used as reference by generating user-defined contrasts. The contrast thus computes difference scores of the dependent variables during each session compared to nighttime (the reference). An interaction effect indicates in this context that groups differ in the manner they sleep/perform during a specific daytime session, compared to nighttime. It thereby allows to explore our main hypothesis that compared to non-nappers, nappers are characterized by a less distinct allocation of sleep during night- compared to daytime and/or a higher impact of day- to nighttime transitions on vigilance and sleepiness levels, respectively.

Family and link functions were applied according to the distribution of the dependent variable (beta distribution for N1%, N3%, and SE, Gaussian distribution for N2% and KSS, log-normal distribution for vigilance measures, zero-inflated Poisson distribution for REM sleep). Finally, circadian amplitude of melatonin expression (height of the fitted waveform), phase and phase angle were compared between groups. For all analyses, sex and age were added as covariates. For group comparisons of melatonin-derived circadian amplitude, baseline levels (fitted baseline function) was further added.

Where relevant, the statistical threshold was Bonferroni-corrected according to the number of models performed by output category ($p < 0.016$ for melatonin, $p < 0.01$ for nap sleep, $p < 0.006$ for night sleep, $p < 0.01$ for vigilance measures, $p < 0.05$ for demographics and actimetry scores reported in Table 1).

Results

Demographics and actimetry-derived daytime rest characteristics

Demographical variables according to group are summarized in Table 1. Nappers and non-nappers did not significantly differ with respect to age, sex, educational level, self-reported perceived sleep quality (PSQI), morningness–eveningness (MEQ), as well as depression (BDI) and anxiety (BAI) scores. However, nappers presented a significantly higher BMI compared to non-nappers (Welsh 2-sample t-test, $t = -2.06$, $p < 0.05$). Nappers also felt significantly sleepier (ESS scale, $t = -3.01$, $p < 0.05$) and presented higher seasonality scores (SPAQ, Wilcoxon rank sum test, $W = 261.5$, $p < 0.01$), compared to non-nappers. Finally, the groups did not significantly differ with respect to overall cognitive status (MMSE and PACC-5 scores).

As expected, extraction of daytime rest characteristics from actimetry recordings revealed that nappers presented significantly increased daytime rest frequency (Wilcoxon rank sum test, $W = 84.50$, $p < 0.0001$) and duration (Welsh 2-sample t-test, $t = -3.59$, $p < 0.0005$) compared to non-nappers (see also Figure 1A). Daytime rest timing did not significantly differ between nappers and non-nappers for which at least one daytime rest period was detected across the recording. Finally, overall locomotor activity over daytime did not significantly differ between nappers and non-nappers.

Melatonin

Group-averaged melatonin profiles (nappers vs. non-nappers) are represented in Figure 2A. Ratio from baseline levels during day 1 (+4 hours to + 10h hours of scheduled wake-up times from the baseline night) are plotted as inset. Groups did not significantly differ in circadian phase (DLMOn) and phase angle. When taking

into account baseline levels (fitted baseline function as a covariate), nappers presented a significantly reduced fitted melatonin amplitude compared to non-nappers (β = 0.42, $p = 0.003$). Note that group differences in fitted amplitude did not reach statistical significance without taking into account baseline levels (β = 0.31, $p = 0.06$).

Nighttime sleep

Nighttime sleep stage characteristics, as extracted from the baseline night are summarized in Table 2. Nappers and non-nappers did not significantly differ with respect to sleep and wake-up times (Table 1), nor did they significantly differ with respect to TST, SE, wake after sleep onset, N2%, N3%, and REM% or sleep latencies to N1 and REM sleep. However, nappers presented increased N1% during nighttime sleep, compared to non-nappers (β = –0.02, $p = 0.004$). Besides, small effects of the factor age were observed for N1% (β = 0.001, $p = 0.03$), REM% (β = 0.003, $p = 0.02$), and sleep latencies to REM sleep (β = –0.024, $p = 0.03$)—all not surviving Bonferroni correction. Sex effects were observed for latency to N1 (β = 0.42, $p = 0.046$—not surviving Bonferroni correction) and to REM (β = 0.35, $p = 0.007$), as well as for N2% (β = 0.041, $p = 0.034$—not surviving Bonferroni correction).

Sleep over the multiple nap protocol

SE and REM% extracted from the multiple nap opportunities are represented in Figure 2B (see Supplementary Figure S1 for all sleep stages). As compared to nighttime nap opportunities, SE was reduced when sleep opportunities were scheduled during the biological day, and particularly around the end of the day (β = –0.33, $p = 0.049$ for time since DLMOn 0h—not

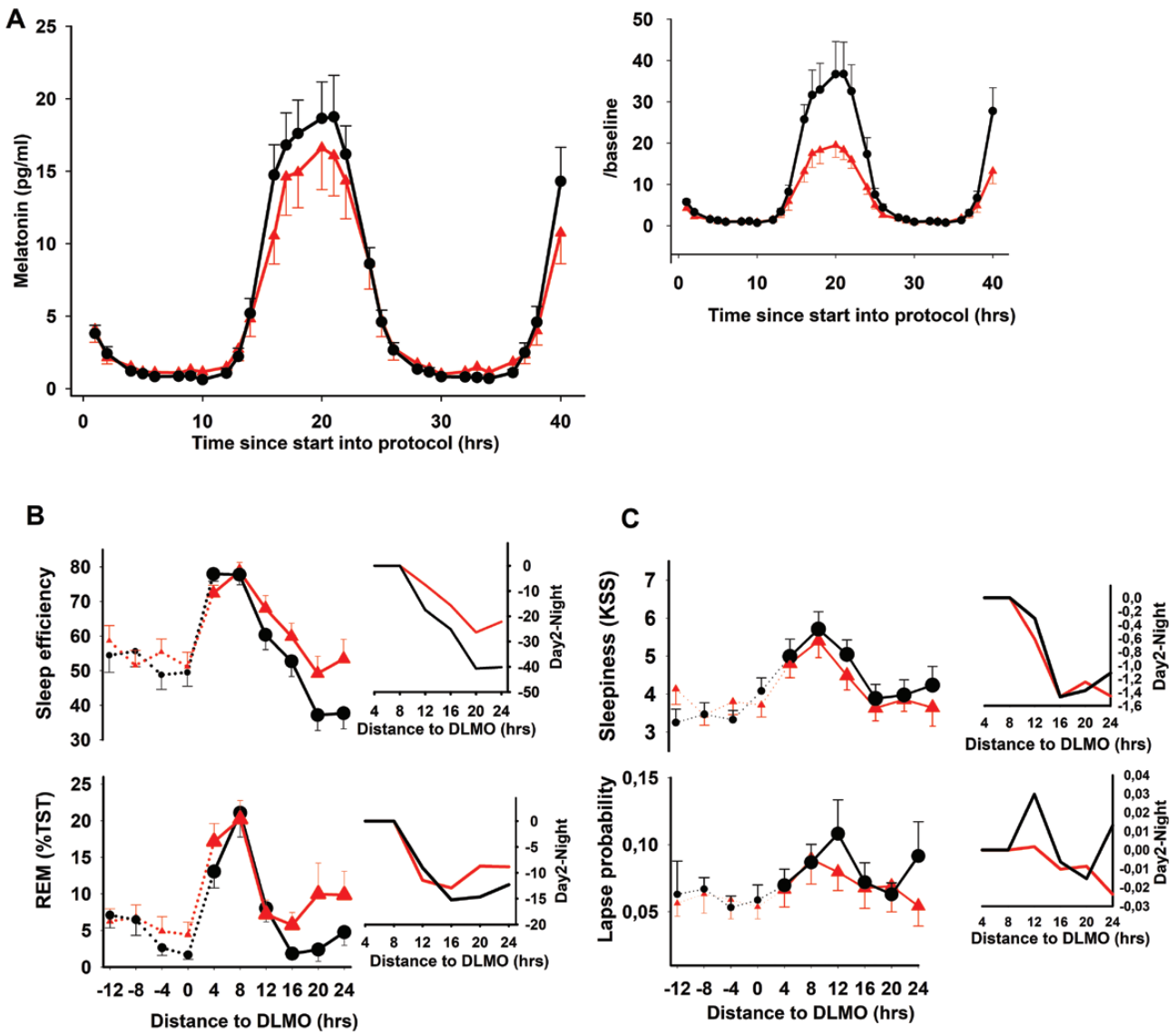


Figure 2. (A) Melatonin profile over the 40-hour multiple nap protocol according to nap group. The inset depicts normalized curves for baseline levels (expressed as ratio from levels + 4 hours-+10 hours after wake-up from day 1). Time course of sleep (B), self-reported sleepiness and vigilance (C) variables aligned to DLMOn over the 40 hours protocol according to nap group. Line plots on the right visualize absolute differences of the daytime sessions compared to the mean of the 2-night sessions (time since DLMOn + 4 hours, +8 hours, used as references in the treatment contrasts of the statistical model). Triangles (red): nappers, circles (black): non-nappers. Note that data acquired during the first day (small dots, dotted line) may be masked by confounds compared to those performed during the second day (large dots, straight line), which should more accurately reflect the individual's circadian sleep–wake promoting drive.

Table 2. Nighttime Sleep Stage Characteristics (Mean and Standard Deviations) by Group

	NAP	NON-NAP	*p*
TST (min)	385.83 ± 39.68	374.95 ± 46.02	
SE (%)	80.3 ± 7.5	79.7 ± 9.2	
WASO (min)	68.68 ± 30.64	64.68 ± 42.24	
Stage 1 (%TST)	8.2 ± 2.1	6.3 ± 2.5	**0.001**
Stage 2 (%TST)	51 ± 8.3	52 ± 8.2	
Stage 3 (%TST)	18.9 ± 4.1	18 ± 6.6	
NREM sleep (%TST)	77 ± 6.1	77 ± 7.4	
REM sleep (%TST)	22 ± 5.9	21 ± 7.3	
SL1 (min)	23.11 ± 23.89	26.2 ± 27.53	
RL (min)	82.03 ± 52.75	79.71 ± 32.87	

TST, total sleep time; SE, sleep efficiency; WASO, wake after sleep onset; SL1, sleep latency to stage 1; RL, REM latency.

surviving Bonferroni-correction; β = –0.59, $p = 0.0003$ for time since DLMOn + 20 hours). Furthermore, an interaction with the factor group revealed that, compared to non-nappers, the difference between night- and daytime SE was reduced in nappers at time since DLMOn + 24 hours (β = 0.79, $p = 0.0009$). N1% was modulated by session, with significantly higher values during the first daytime nap in the morning hours after wake-up from the baseline night (time since DLMOn –12 hours, β = 0.55, $p = 0.0001$) compared to nighttime naps. No differences, nor interaction was observed with the factor group. N2% did not significantly differ for naps scheduled during daytime, compared to nighttime sleep opportunities, nor was it modulated by the factor group. N3% was significantly lower during naps in the second half of the first day, compared to nighttime naps (time since DLMOn –12 hours, –8 hours; β = –0.45, –0.47, respectively all *p*s < 0.005), but no main effect, nor significant interaction with the factor group was observed. REM% was significantly higher during nighttime naps, compared to daytime naps (for time since DLMOn –4, 0, +12, and + 24 hours, β = –0.65, –0.53, –0.40, –0.46, respectively, all ps $p < 0.0001$). Furthermore, a significant main effect of group (β = –0.71, $p = 0.001$), as well as a significant interaction between group and session (for time since DLMOn –4, 0, +12, and + 20 hours, β = –0.65, –0.38, 0.57, –1.01, respectively, all *p*s < 0.0001) indicated that day–night differences in REM% were reduced in nappers, compared to non-nappers (see Figure 2B). Note that a significant effect of age and sex was observed for N3%, such that reduced N3% over all circadian phases was associated with increasing age and with being male (β = –0.18 and –0.22, respectively, all *p*s < 0.005). In addition, increasing age was associated with higher sleep efficiencies over the multiple nap protocol (β = 0.13, $p = 0.027$—not surviving Bonferroni correction), while men presented lower overall sleep efficiencies (β = –0.28, $p = 0.022$—not surviving Bonferroni correction).

Self-reported sleepiness and psychomotor vigilance performance

Sleepiness, as assessed by the KSS, was modulated over the 24-hour cycle with significantly increased sleepiness levels during the biological night, compared to daytime assessments (for time since DLMOn –8, +12, +20 hours, β = –0.63, –0.65, –0.44 respectively, all *p*s < 0.01, Figure 2C). Furthermore, a significant interaction effect revealed that day- to nighttime differences in sleepiness were higher in non-nappers, compared to nappers, but only for the sleepiness assessment following the baseline night (time since DLMOn -12 hours, β = 0.63, $p = 0.0078$). For psychomotor vigilance performance, modulations over the 24-hour cycle were observed, with better performance (i.e. reduced lapse probability) towards the end of the second day (time since DLMOn + 24 hours, β = –0.13, $p = 0.0086$) compared to nighttime assessments. Finally, an interaction with the factor group indicated that day- to nighttime differences were higher in non-nappers, compared to nappers at time since DLMOn + 12 hours (β = 0.14, $p = 0.013$—not surviving Bonferroni correction) and at time since DLMOn + 24 hours (β = 0.26, $p = 0.00047$). As depicted in Figure 2C, lapse probabilities of non-nappers significantly increased during this session, compared to their nighttime performance, while day–night modulations were not significant in nappers. A follow-up analysis revealed that a similar pattern was observed for median RTs, the mean of 10% of fastest and the mean of 10% of slowest RTs (interaction with group at DLMOn + 24 hours: β = 0.13, $p = 0.007$, β = 0.03, $p = 0.08$—not surviving Bonferroni correction and β = 0.17, $p = 0.004$, respectively). Note finally that a significant main effect of sex and age was observed for lapse probabilities (and the other metrics—values not reported), such that, women had higher lapse probabilities than men (β = 0.24, $p = 0.00019$) and age was positively associated with lapse probabilities (β = 0.076, $p = 0.012$—not surviving Bonferroni correction).

Discussion

Our main results indicate that along with a reduced circadian amplitude in melatonin secretion, healthy older nappers are characterized by reduced day–night differences in SE and REM sleep, compared to their non-napping counterparts. These results suggest altered circadian regulation of sleep as a cause or consequence of chronic napping in the aged and thereby contribute to the understanding of nap regulation during healthy aging.

In apparent contrast to the reported effects of napping as an efficient countermeasure to sleep debt, chronic napping is increasingly advertised as a health risk factor in the context of aging. Napping and associated characteristics, including duration and frequency, have been related to medical comorbidities and increased mortality [19, 20, 57], cognitive decline [23, 24, 29], but also to the prognosis and progression of Alzheimer's disease [26]. These observations are not necessarily incompatible with the previously reported beneficial effects of napping, considering that the reasons underlying napping can critically evolve across

lifespan, so are the approaches used to study napping in different contexts (e.g. spontaneous adoption of napping in the field in most epidemiological studies vs. assessing the acute effects of napping as a countermeasure for sleep loss or daytime sleepiness in the laboratory). In our study, we prospectively recruited individuals according to their napping habits in the field and assessed its potential impact on circadian sleep regulation in healthy older individuals. In the context of the aging brain, we assumed that chronic napping reflects sleep–wake cycle fragmentation and altered underlying circadian sleep regulation.

From a demographical point of view, our groups were matched with respect to age, sex, and educational status and did not suffer from signs of depression and anxiety, thereby limiting potential confounds of these variables on our output measures. However, nappers presented a significantly higher BMI compared to non-nappers. This finding may be put in line with a recent report of a positive association between nap duration and risk of obesity [19] and opens the question of whether reduced physical activity and/or calorie consumption is associated with chronic napping. Overall locomotor activity as extracted from actimetry did, however, not differ between our groups, but future studies could follow-up on this finding by assessing the balance between calorie consumption and more precise measures of physical activity. With respect to sleep-related scores, nappers, and non-nappers did not differ in their self-reported perceived sleep quality nor in morningness–eveningness scores indicating that napping behavior and associated differences in actimetry-derived daytime rest characteristics do not solely result from differences in self-reported perceived nighttime sleep quality or sleep timing preference. Furthermore, except for increased N1%, we did not observe any group differences in sleep stage parameters assessed during the baseline night preceding the multiple nap protocol. Enhanced N1% may however be indicative of a lighter sleep, potentially more prone to disruption in nappers. Within this context, we previously observed that increased daytime rest frequency as assessed with actimetry is associated with a more fragmented rest towards the end of the night [29]. We also observed that individuals who rest later in the day (putatively surrounding the wake-maintenance zone) go to bed later with respect to their circadian phase, thereby indicating circadian misalignment.

From a sleep regulatory point of view, a consensus on the mechanisms underlying changes in the structure and timing of sleep across lifespan has not yet been reached. It has, however, been suggested, that they can be suitably reflected by a concomitant reduction in the wake-dependent homeostatic build-up of sleep pressure and a reduced circadian wake propensity drive [10]. Here, we focused on the modulatory potential of chronic napping on circadian sleep–wake propensity. We first observed that self-reported perceived daytime sleepiness, as assessed by the Epworth Sleepiness Scale, was higher in nappers, compared to non-nappers. This may indicate heterogeneity in the processes underlying age-related changes in sleep regulation, depending on whether or not, the individual adopts a chronic napping habit. Second, altered circadian wake propensity would lead to a less distinct allocation of sleep into the biological night and of wakefulness during daytime. Within this context, neuronal loss in the SCN has been previously associated with reduced circadian rhythm amplitude of locomotor activity in older adults [58] and alteration at this level may be particularly determinant for chronic napping. Accordingly, our data indicate that sleep initiation and/or maintenance become facilitated during the active wake period in chronic nappers, compared to their non-napping counterparts.

Experiments in which the sleep–wake cycle was desynchronized from endogenous circadian rhythms revealed quantitative age-related differences in circadian sleep regulation. The timing of circadian rhythms, such as the core body temperature and melatonin rhythm, is advanced [7], total sleep duration is reduced at all circadian phases and older people are more susceptible to the negative effects of circadian phase misalignment than young adults [59–61]. Concomitantly, a reduced age-related amplitude of circadian rhythmicity in endogenous core body temperature [5] and melatonin has been identified in some [9, 62, 63] but not all [64] studies. By prospectively recruiting participants with respect to their napping habits, we observed that healthy older nappers presented a reduced amplitude in the 24-hour melatonin profile compared to non-nappers. This finding not only speaks in favor of reduced circadian amplitude and associated altered circadian sleep–wake promotion (as observed in nap sleep measures) in nappers, but also underlines that the absence or presence of chronic napping should be reported as it may at least partially explain the mixed results when assessing age-related changes in circadian markers. Note as well that group differences in circadian amplitude of melatonin secretion reached significance when taking into account the fluctuations in daytime baseline levels. Sleep can mask melatonin expression [65]. It is thus possible that enhanced facility in initiating and/or maintaining sleep during daytime affects or is affected by melatonin expression. Animal studies also suggest that SCN integrity plays a role for limiting the secretion of melatonin during the biological night [66].

There has been some question on whether age merely affects the wake-consolidating function of the circadian system, that is, the promotion of wakefulness, or its sleep-consolidating function, namely the active promotion of sleep during the early morning hours, at the end of the habitual sleep phase. A forced desynchrony study reported that sleep latencies were rather similar between age groups throughout the circadian cycle, even though the shortest sleep latencies located around the temperature nadir were somewhat longer in the older [8]. Concomitantly, it was observed that sleepiness and alertness levels were similar in old and young adults throughout the waking period [67], indicating no major changes in the amplitude of the circadian modulation of wake maintenance, combined with a possible reduction of the circadian drive for sleep in the early morning hours. Furthermore, when changes in daytime sleepiness were assessed by the Multiple Sleep Latency Test, lower values were observed with increasing age [68]. However, when using a multiple nap protocol, similar to the one applied here, Münch and colleagues observed that self-reported sleepiness ratings and the amount of sleep occurring during the so-called “wake maintenance zone” [69] in the late afternoon was higher in older than in young adults [9]. Similarly, previous findings from a nap-study with short sleep–wake cycles [70] reported that older participants exhibit a higher sleep propensity (reflected in TST) during the naps occurring during maximal circadian wake promotion. Here, we observed that daytime and nighttime differences in the ability to sleep (SE) were reduced in nappers, compared to non-nappers, suggesting that nappers were more able to initiate and/or maintain sleep at adverse circadian phase (i.e. during the active wake period). Interestingly, this difference reached statistical significance when the sleep opportunity was scheduled to a time window surrounding the wake-maintenance zone (time since DLMOn + 24 hours), characterized by highest circadian wake promotion. At contrast, even though not directly statistically compared, nap sleep during the biological night appeared not necessarily affected (see Figure 2b). Combined, these findings indicate that chronic napping, the

incidence of which increases with age, is hallmarked by a reduction in circadian wake promotion. Besides SCN-orchestrated circadian sleep–wake promotion, the orexin/hypocretin neurons have been identified as a key actor for wake promotion, but also as a state-stabilizing system [71]. Orexinergic deficiency has been associated with narcolepsy, a sleep disorder characterized, amongst others, by excessive daytime sleepiness and an irresistible need to sleep during the day. Altered REM sleep regulation, as well as dislocation of REM sleep have also been suggested to be characteristic for this sleep disorder [72].

Notably, group-by-session interactions were most pronounced for REM sleep expression, with nappers showing less pronounced day–night differences, compared to their non-napping counterparts. Besides its role in sleep timing, the circadian system also regulates sleep structural aspects. Amongst them, REM sleep has been shown to be under the strongest circadian control [73] and the circadian pacemaker has been suggested to actively promote REM sleep at specific times of the day [74, 75]. Interestingly, a reduced modulation of REM sleep has been observed in older, compared to young individuals [9, 51]. Our results provide first evidence that napping in the aged leads to or may be the consequence of a disproportional reduction in the circadian REM sleep propensity drive, leading to an overall higher REM sleep expression, when prompted to sleep during the active wake period.

Note that for both, SE and REM sleep, the effects were restricted to the second biological day which may accurately reflect the individual's sleep–wake-promoting drive. Results acquired on the first day may indeed be masked by confounds, including the habituation to the imposed sleep–wake regime, group differences in the ability to get habituated to such regime, potential interindividual differences in nighttime sleep parameters or unmet sleep needs which may be washed out during the first naps of the protocol.

With respect to the 24-hour modulation of vigilant attention, higher performances were observed when tested during day- compared to night-time. Notably, reduced performance, both at the beginning and the end of the biological day- compared to nighttime, was observed in non-nappers, but not in nappers. While this finding indicates enhanced modulation over the 24-hour cycle in non-nappers, it appears at first glance contradictory to the above suggested enhanced circadian wake promoting drive underlined by reduced sleep abilities in the evening hours. Note that the interpretation of these findings is complex, considering that performance levels are likely affected by sleep parameters of the preceding nap opportunities which appeared to differ. Future studies should assess how and to which extend nap sleep structure affects subsequent vigilance levels over the 24-hour cycle.

Limitations and future directions

Our study was not designed to disentangle the respective influence of sleep homeostatic and circadian processes on sleep–wake cycle organization. As these processes interact, it would have been interesting to assess sleep parameters over the 24-hour cycle, but under varying sleep pressures levels. In the same vain, it would also be relevant to assess potential differences in microstructural aspects of both night- and daytime sleep, such as sleep slow wave parameters, sleep spindle composition or spectral markers of REM sleep.

Future research should assess the impact of napping on the neurobiological substrates, underlying sleep and wake promotion, and further assess the functional relevance of this phenotype for age-related changes in structural and functional brain integrity. First indications of our work go indeed in the direction that circadian REM sleep regulation affects regional macrostructural integrity (i.e. cortical gyrification) in the aging brain [51].

Furthermore, the hypothesis of altered wake state stability could be addressed from a more cognitive point of view, by assessing the impact of napping on performance over different cognitive domains and according to task characteristics (e.g. time on task effects, modulation of cognitive load). Indeed, while our groups did not differ with respect to overall cognitive status as assessed by the PACC-5 composite score, exploring performance on a wider range of cognitive domains would allow us to assess more subtle group differences. According to previous literature reports [23, 29], these may be particularly observed in tasks assessing episodic memory.

Here, we prospectively recruited our samples with respect to their napping phenotype. The two groups (nappers vs. non-nappers) were further matched with respect to a series of demographic variables and thoroughly screened with respect to the health status and assessed in a controlled laboratory routine to avoid the influence of environmental factors that may differ between individuals/groups. It is thus reasonable to assume that observed differences in our main variables of interest are attributable to differences in the nap phenotype. Note that we also observed group differences in actimetry-derived daytime rest characteristics, further corroborating that the groups differed in the amount of resting behavior during the active daytime period. It has to be noted, however, that actimetry-derived daytime rest does not reflect absolute daytime sleep as such, even though the latter was observed to significantly correlate with napping as derived from sleep diaries. A bias between the detection of rest versus sleep with actimetry may further depend on varying levels of activity, including for example sedentary wakefulness.

Finally, the beneficial versus disadvantageous effects of napping largely depend on the context (e.g. developmental, cultural, reasons underlying napping, and nap intentionality). As such, also in the context of the aging brain, napping may be used for different reasons and thereby increase the heterogeneity of our sample with respect to its underlying biological, social, or behavioral correlates. This could be potentially assessed in the future by applying cluster analyses based on nap characteristics in a larger sample.

Conclusion

From a circadian perspective, the global picture that emerges from our findings suggests that chronic napping in the aged is associated with altered circadian regulation, potentially by affecting its wake-consolidating function during the daily active phase. This is exemplified by the nap phenotype as such (enhanced intrusions of rest bouts into the active wake period during everyday life), but also by the increased ability to maintain and/or initiate sleep and more particularly REM sleep during the biological day.

Supplementary Material

Supplementary material is available at *SLEEP* online.

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Data Availability Statement

The data underlying this article will be shared on request to the corresponding author.

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