Chronobiology International, 23(1&2): 461–474, (2006)
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ISSN 0742-0528 print/1525-6073 online
DOI: 10.1080/07420520500545813

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AGE-RELATED CHANGES IN THE CIRCADIAN AND HOMEOSTATIC REGULATION OF HUMAN SLEEP

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The reduction of electroencephalographic (EEG) slow-wave activity (SWA) (EEG power density between 0.75–4.5 Hz) and spindle frequency activity, together with an increase in involuntary awakenings during sleep, represent the hallmarks of human sleep alterations with age. It has been assumed that this decrease in non-rapid eye movement (NREM) sleep consolidation reflects an age-related attenuation of the sleep homeostatic drive. To test this hypothesis, we measured sleep EEG characteristics (*i.e.*, SWA, sleep spindles) in healthy older volunteers in response to high (sleep deprivation protocol) and low sleep pressure (nap protocol) conditions. Despite the fact that the older volunteers had impaired sleep consolidation and reduced SWA levels, their relative SWA response to both high and low sleep pressure conditions was similar to that of younger persons. Only in frontal brain regions did we find an age-related diminished SWA response to high sleep pressure. On the other hand, we have clear evidence that the circadian regulation of sleep during the 40 h nap protocol was changed such that the circadian arousal signal in the evening was weaker in the older study participants. More sleep occurred during the wake maintenance zone, and subjective sleepiness ratings in the late afternoon and evening were higher than in younger participants. In addition, we found a diminished melatonin secretion and a reduced circadian modulation of REM sleep and spindle frequency—the latter was phase-advanced relative to the circadian melatonin profile. Therefore, we favor the hypothesis that age-related changes in sleep are due to weaker circadian regulation of sleep and wakefulness. Our data suggest that manipulations of the circadian timing system, rather than the sleep homeostat, may offer a potential strategy to alleviate age-related decrements in sleep and daytime alertness levels.

Keywords Aging, Sleep, Melatonin, Sleep-deprivation protocol, Nap protocol, Constant routine, EEG spectral analysis, Wake-maintenance zone

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INTRODUCTION

Increased awakenings and reduced slow-wave sleep (SWS) are the hallmarks and most consistent findings in the literature of age-related sleep changes. Even extremely healthy older people usually spend about twice as much time during the night in unwanted wakefulness than do younger adults (Bliwise, 1993). This suggests that the impairment in sleep consolidation with age is associated with an aging process *per se*, rather than being secondary to other ailments associated with aging. Moreover, these changes do not suddenly appear at age 60 yrs, but gradually start to occur during the middle years of life (Carrier et al., 2001). Whether sleep disruption with age comes about as a result of alterations in the circadian or the homeostatic facet of sleep regulation, or both, remains unclear.

A review of the current literature reveals a predominance of circadian rhythm-related changes with age. These findings, however, are far from being consistent. Most (Weitzman et al., 1982; Van Coevorden et al., 1991; Czeisler et al., 1992), but not all, studies report a decline in the amplitude of circadian markers, such as core body temperature (CBT), melatonin, and cortisol (Zeitzer et al., 1999; Niggemyer et al.; 2004; Monk, 2005). The elderly usually have earlier habitual bed and wake times (Duffy et al., 1998; Duffy and Czeisler, 2002), but this is not always paralleled with an advance in circadian phase or phase angle differences, when compared to the young (Carrier et al., 1999; Kripke et al., 2005; Monk, 2005). On the other hand, recent evidence suggests that the endogenous period of the circadian pacemaker (CBT tau) of young and older individuals is remarkably similar at $\sim$24.2 h (Czeisler et al., 1999). Thus, the earlier circadian phase position seen in older volunteers is probably not attributable to any age-related change in the endogenous circadian period.

To add to the complexity of circadian findings, the circadian period has not been found to correlate significantly with wake time, circadian phase, and diurnal preference (morningness-eveningness) in the elderly as it does in young subjects (Duffy and Czeisler, 2002). These findings indicate that the circadian rhythms of older people are in some way dysfunctional, but whether this is related to reduced sleep consolidation, altered sleep-wake timing, or reduced SWS with age remains unclear. According to Carrier and colleagues (1999), decrements in sleep consolidation associated with healthy aging do not appear to be related to changes in the phase-angle difference between the output signal from the circadian timing system and sleep. Furthermore, work of Dijk and colleagues indicates that the decrease in sleep consolidation in older volunteers seems to occur independently of circadian phase (Dijk et al., 2001), and that SWS is reduced at all circadian phases (Dijk et al., 1999). According to these authors, the deterioration of sleep continuity appears to be related primarily to a reduction of non-rapid eye movement (NREM) sleep consolidation. In

other words, the protective effect of the NREM sleep stages 3 and 4 on the probability of awakening is reduced in the elderly. However, the inability to sustain longer sleep bouts within a bedrest episode does not necessarily imply a weaker homeostatic influence in elderly individuals. It could well be that the degree of synchronization of thalamocortical oscillations is reduced with age, independent of possible alterations in the homeostatic process.

QUANTIFYING CIRCADIAN AND SLEEP-WAKE HOMEOSTATIC PROCESSES

Early studies indicated that sleep homeostasis, the sleep-wake-dependent regulation of sleep, cannot solely account for changes in sleep propensity. Total sleep loss was only compensated by a ~20% increase in sleep duration during the following recovery night in humans (Patrick and Gilbert, 1896 as cited by Gulevich et al., 1966). This led to the assumption that, besides the homeostatic process, at least one other process must be involved in the regulation of sleep duration. In fact, it was realized that such variations in sleep duration occur in a consistent and predictable manner that depends on the time of day when subjects go to sleep (Czeisler et al., 1980; Zulley, 1980; Strogatz et al., 1986). The role of 'time of day,' referred to as the circadian process C, and the homeostatic process S have been conceptualized in the two-process model of sleep regulation as a means to predict sleep propensity in humans (Borbély, 1982; Daan et al., 1984). According to this model, the timing of sleep and wakefulness is determined by the interaction of the circadian process C, generated by the endogenous circadian clock in the suprachiasmatic nuclei (SCN), and the homeostatic process S.

It was recognized early on that, for a better understanding of the mechanisms underlying the timing of the sleep-wake cycle, a distinction should be made between internal and environmental factors that both contribute to variations in the propensity to initiate and terminate sleep. Nathaniel Kleitman (1987) was the first to conduct an experiment in which human beings were studied in the absence of periodic time cues in the external environment. He realized that in order to prove the existence of internal time or the existence of endogenous self-sustained rhythms, paradigms must be applied in which it is possible to desynchronize internal time from external time. In the Mammoth Cave in Kentucky (USA) in 1938, he scheduled subjects to live on artificial day-lengths that deviated from 24 h. Under such conditions, near-24 h rhythms (circadian) were unable to entrain to the newly imposed day length, but they continued to oscillate with their endogenous period. It was possible to separate the influence of the timing of the sleep-wake schedule from that of the circadian

pacemaker. This imposed desynchrony between the sleep-wake schedule and the output of the circadian pacemaker occurs only under conditions in which the non-24 h sleep-wake schedule is outside the range of entrainment or range of capture of the circadian system. This protocol was termed the forced desynchrony (FD) protocol.

In 1967, Aschoff and coworkers (1967) convincingly demonstrated the human sleep-wake cycle can also spontaneously desynchronize from the core body temperature cycle. The period of core body temperature rhythm remains rather stable during spontaneous internal desynchronization. The period of the sleep-wake cycle, however, is unstable and may vary within a subject, from being close to the period of the temperature rhythm to close to twice the period of the core body temperature rhythm (circabidian).

One key observation in the spontaneous desynchrony protocol has been that sleep is rarely initiated on the latter part of the rising portion of the core body temperature 24 h rhythm. This window has been called the "wake maintenance zone" (Strogatz et al., 1987). FD protocols have been implemented to further quantify the interactions between the sleep-wake cycle and circadian processes in the regulation of sleep, the EEG during sleep and wakefulness, and neurobehavioral and physiological variables (Dijk and Czeisler, 1995; Hiddinga et al., 1997; Wyatt et al., 1999; Cajochen et al., 2002). In these protocols, scheduled sleep and wake episodes occur at virtually all circadian phases, and when light intensities during scheduled waking episodes are kept low, the pacemaker free-runs with a stable period in the range of 23.9 to 24.5 h (Czeisler et al., 1999). Furthermore, as subjects are scheduled to stay in bed in darkness, the variation in the amount of wakefulness preceding each sleep episode is minimized. It is thus possible to average data either over successive circadian cycles or over successive sleep or wake episodes and to thereby separate these two components. This averaging process, by subtracting background noise which is not temporally related to the evoked component, serves to isolate the circadian profile of the variable of interest by removing the part of the confounding sleep-wake-dependent contribution or *vice versa*. The efficacy of the FD protocol in removing or uniformly distributing several driving factors is demonstrated by the observation that the observed period of the pacemaker was nearly identical in FD protocols with markedly different cycle lengths, for example: 11, 20, 28, or 42.85 h, and with markedly different levels of physical activity (Dijk and Czeisler, 1995; Hiddinga et al., 1997; Wyatt et al., 1999; Wyatt et al., 2004).

ARE CIRCADIAN AND/OR SLEEP HOMEOSTATIC ASPECTS OF SLEEP REGULATION CHANGED WITH AGE?

Age-related changes in sleep structure persisted during desynchrony of the sleep-wake cycle and the rhythms of core body temperature and

melatonin in FD protocols. At no circadian phase was sleep efficiency and SWS in older people restored to the values obtained in young subjects during entrainment (Dijk et al., 1999). On the circadian side, Dijk and colleagues (1999) reported an age-related reduction in the circadian amplitude of the CBT rhythm of ~20% to 30% which was concomitant with a ~1 h phase advance of the CBT and melatonin rhythms. However, these pronounced circadian alterations and reported circadian changes in sleep consolidation and sleep spindle activity did not appear to be associated with the observed age-related changes in sleep. Dijk and coworkers (2000) concluded that those changes may be related to the sleep process, itself, and not to changes in circadian period or reduction of the circadian drive for wakefulness, although the circadian drive for sleep in the morning was somewhat impaired in the elderly. On the homeostatic side, FD studies confirmed the shallower decline in SWS and SWA across the night shown in earlier studies (Dijk et al., 1989; Landolt et al., 1996). This attenuation in the slope of the SWS/SWA decline was most likely due to more interrupted sleep in the second half of the night in the older volunteers (Dijk et al., 2000). Whether this finding can be explained by a reduced homeostatic drive, the reduction in circadian drive promoting sleep in the second half of the night, or altered interaction of the circadian and homeostatic process is not clear.

FD studies cannot really prove whether there is a reduction in sleep homeostatic drive in the elderly, since prior wakefulness is not manipulated and remains rather stable in a certain range across the entire FD protocol. Therefore, we attempted to study older people in protocols in which we challenged the sleep homeostat by either an extension (high sleep pressure) or a reduction of prior wakefulness (low sleep pressure) while assessing circadian phase and amplitude under posture-controlled conditions.

In the high sleep pressure protocol, we sleep-deprived (SD)16 young (20 to 31 yrs) and 16 older (57 to 74 yrs) volunteers for 40 h under constant routine conditions (CR, for details, see Cajochen et al., 2001); whereas, the same volunteers were sleep satiated for 40 h in the low sleep pressure protocol by scheduling them to 10 alternating cycles of 150 min of wakefulness followed by 75 min of sleep (Nap) also under CR conditions. To exclude any sequence effect of the protocols, the order of the SD and Nap protocols were counterbalanced. Furthermore, to exclude differences in phase preference, the young and older subjects were matched according to chronotype by means of a morningness-eveningness questionnaire.

In both protocols, the secretion of salivary melatonin was reduced in the older study participants, but there was no significant difference in the phase position of the circadian melatonin rhythm or timing of the sleep-wake cycle, and nor did the phase angle between them differ (Knoblauch et al., 2005; Münch et al., 2005). The reduction in melatonin

secretion in our study was ~30%, which is in good agreement with the reported age-related circadian amplitude reduction in the FD study (Dijk et al., 2000). We also confirmed the findings of earlier studies (Dijk et al., 1989; Landolt et al., 1996; Carrier et al., 2001; Niggemyer et al., 2004); compared to the young, even very healthy older volunteers have less consolidated sleep and less SWS and SWA during baseline as well as during recovery sleep from both the SD (Münch et al., 2005) and Nap protocol (Münch et al. submitted). However, the sleep homeostat in our older subject sample was still operational and showed similar relative SWA-responses to both high and low sleep pressure, with the exception being that older volunteers exhibited a significantly shallower decline in sleep homeostatic pressure as indexed by relative SWA, particularly in the frontal brain regions after SD (Münch et al., 2004). In fact, when averaged over the entire night, the frontal predominance in the homeostatic SWA response to high sleep pressure disappeared in the older volunteers (Figure 1). This finding provides quantitative evidence for the hypothesis that the frontal brain regions are particularly vulnerable to the effects of elevated sleep pressure ('prefrontal tiredness') and aging ('frontal aging'). It remains, however, to be elucidated whether this result reflects an age-related deficit of the sleep homeostat in brain areas most vulnerable to the effects of sleep loss, or whether it is related to changes in cortical function with age, which are not linked to

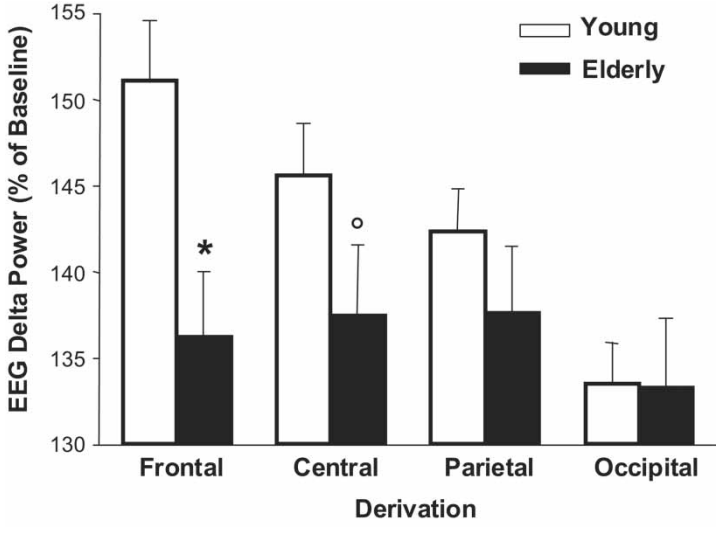

FIGURE 1 Relative electroencephalogram (EEG) delta power density (1.25 to 3.75 Hz) during NREM sleep along the antero-posterior axis (Fz, Cz, Pz, and Oz) in the young (*white bars*) and elderly (*black bars*) study group (mean values + 1 SEM; $n = 16$ for both age groups) after 40 h of sleep deprivation. Values are expressed as percentage of the baseline night; $^{*}p < 0.05$; $^{\circ}p < 0.1$. Adapted with permission from Münch and colleagues (2004).

sleep homeostatic processes, or whether the recently proposed synaptic potentiation rate in frontal brain areas, which is assumed to be high during wakefulness (Tononi and Cirelli, 2003), decreases with age, resulting in a concomitant diminution of SWA increase during the following sleep episode.

We found the strength of the circadian process as indicated by several circadian variables is weakened with age. We have quantitative evidence for a weaker circadian arousal signal in the older group. Significantly more sleep occurred during the wake maintenance zone, and subjective sleepiness ratings in the late afternoon and evening were higher (Münch et al., 2005) (Figure 2). This is in agreement with results of an ultrashort sleep cycle study in which subjects were scheduled to sleep for 7 min and to be awake for 13 min over 24 h (Haimov and Lavie, 1997). In addition to the diminished melatonin secretion in the older group, we also found a reduced circadian modulation of REM sleep together with less pronounced day-night differences in the sleep EEG in the lower alpha and spindle range. Thus, our data indicate that age-related changes in sleep propensity are clearly related to a reduced strength of the circadian signal opposing the homeostatic drive for sleep (Münch et al., 2005). Furthermore, the multiple nap protocol used in conjunction with the CR conditions revealed an age-dependent weaker coupling of the circadian rhythms of spindle frequency and sleep propensity to the circadian rhythm of melatonin secretion (Knoblauch et al., 2005). In fact, the spindle frequency rhythm was markedly advanced relative to that found in the younger age group and relative to the peak in melatonin secretion (Figure 3). The circadian modulation of sleep spindles is such that low-frequency spindles are promoted during the phase of melatonin secretion, *i.e.*, during the usual sleep time, spindle frequency is reduced and EEG power density and spindle amplitude in the low spindle frequency range is increased as compared to the daytime. Therefore, it could be hypothesized that the circadian modulation of spindle frequency is related to a circadian signal that modulates the level of arousal and sleep propensity to facilitate a consolidated sleep bout during the night and a consolidated wake bout during the day.

In our study, the age-related changes in the circadian modulation of sleep spindle frequency were associated with a reduced amplitude of the circadian profile of sleep propensity (see Figure 2). An earlier reduction in spindle frequency in the older volunteers could be related to an attenuation of the circadian arousal signal in the evening. Similarly, an earlier increase in spindle frequency at the end of the night could be associated with the often-reported difficulties for older people to maintain sleep in the morning hours. However, we did not find a clear correlation between spindle frequency and sleep consolidation in the majority of our subjects.

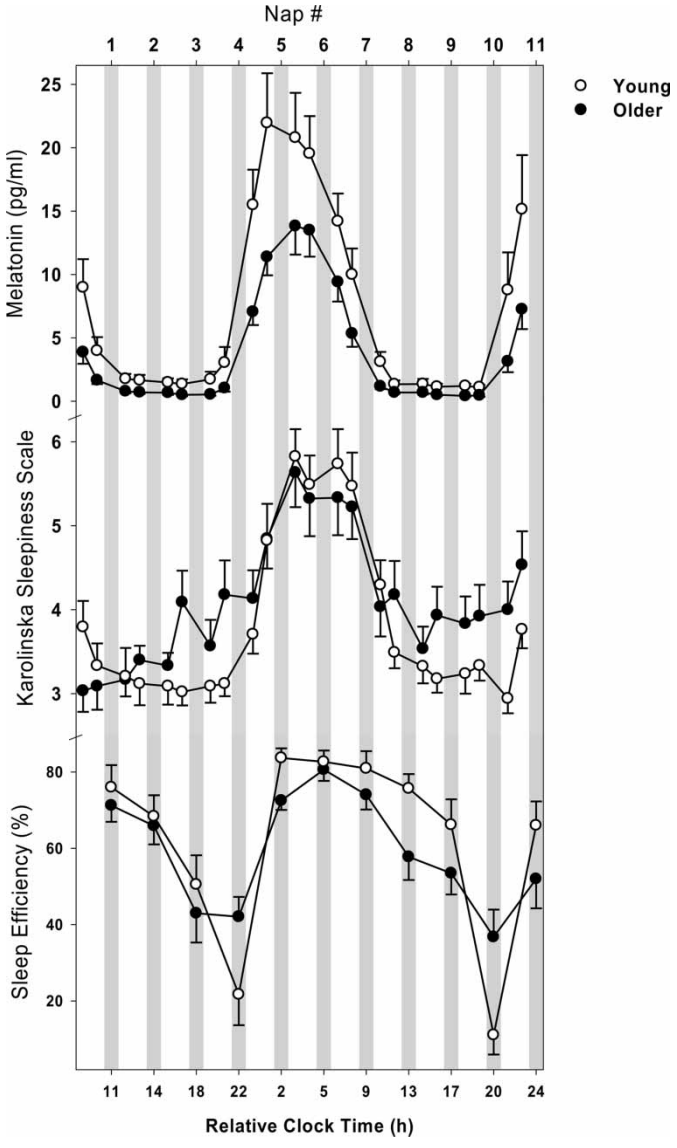

FIGURE 2 The *top panel* shows melatonin secretion during the 40 h nap protocol (150 min of scheduled wakefulness followed by 75 min of scheduled sleep) between young (white circles) and older volunteers (black circles, mean value + or − SEM ($n = 17$ for the young and $n = 15$ for the older groups, respectively). The *middle panel* represents subjective sleepiness ratings (KSS) during scheduled wake episodes and the *bottom panel* represents sleep efficiency during scheduled nap episodes for both age groups. Adapted with permission from Münch and colleagues (2005).

Whereas Dijk and coworkers (2001) interpreted their FD findings of reduced sleep consolidation as reflecting a reduced sleep homeostatic pressure in older people, we interpret our findings as being attributable to the circadian system, since we do not have strong evidence for a

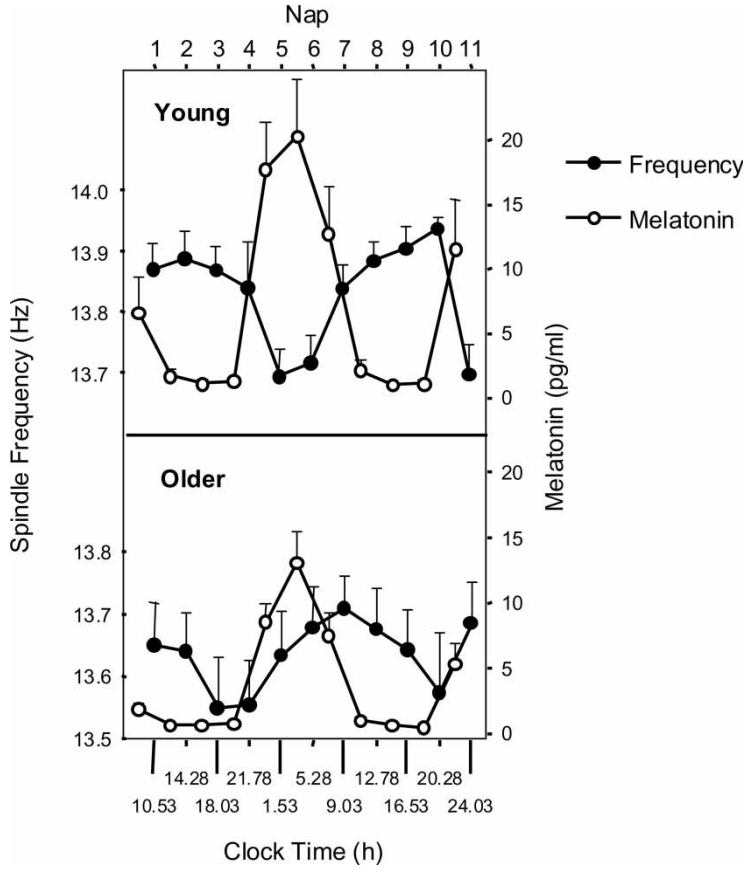

FIGURE 3 Time course of spindle frequency (*filled circles*) and salivary melatonin levels (*open circles*) for the younger (*upper panel*) and the older (*lower panel*) age groups (mean + SEM) during the 40 h nap protocol (150/75 min wake/sleep cycle). For the older age group, 10 or more subjects participated in all naps, and in the younger group, 12 or more subjects participated except for nap 4 ($n = 5$) and nap 10 ($n = 3$). Adapted with permission from Knoblauch and colleagues (2005).

reduction in homeostatic sleep pressure in the older participants as indexed by EEG SWA in response to high and low sleep pressure conditions. Our findings corroborate several studies, which have demonstrated a similar response to SD in both young and older subjects (Carskadon and Dement, 1985; Bonnet, 1986; Brendel et al., 1990). Similarly, two recent studies have found comparable linear trends in low-frequency EEG activity during sustained wakefulness (Drapeau and Carrier, 2004) and across a 90 min protocol during scheduled sleep episodes (Niggemyer et al., 2004) of young and older subject samples. In our view, the circadian profile of sleep consolidation reported by Dijk and coworkers (1999), particularly in the last quarter of the sleep episode, clearly reflects a decreased circadian modulation. According to this profile, the impairment in sleep consolidation between the elderly

and the young completely disappeared in the wake-maintenance zone, while it was greatest in the morning hours. Furthermore, an indication of a 12 h component in the circadian modulation of sleep consolidation became apparent in the older subjects. All of this may reflect a reduced circadian drive in the elderly to promote sleep or wakefulness at the respective times of day. Nonetheless, we think the level of sleep pressure and the non-additive interaction of the circadian and homeostatic processes play an important role in how one interprets age-related changes in sleep as being more related to the circadian or homeostatic facets of sleep regulation.

Although our data favor a diminished circadian amplitude with age, we obviously cannot prove whether SCN activity is decreased or whether the expression of the circadian signal is selectively impaired more "downstream" from the SCN in the sleep-generating system. At least from human post-mortem studies, there is ample evidence for neuronal degeneration of the SCN in senescence, which strongly suggests that the circadian pacemaker in the human brain becomes progressively disturbed during aging (Hofman and Swaab, 2005). The reduced circadian amplitude could also reflect a differential response to "zeitgeber history" (*e.g.*, prior light exposure). We have no evidence from actigraphic data obtained prior to the study that our older study volunteers experienced a different light-dark cycle than the young ones. However, it is certain, due to age-related changes in lens properties (Charman, 2003) and perhaps to other aspects of retinal function, that our older subjects received less short-wavelength light, specifically important for circadian light input (Lockley et al., 2003; Cajochen et al., 2005). This could have led to an age-related reduction in the circadian response to light (Herljevic et al., 2005), resulting in a weaker circadian drive.

Besides the SCN, the ventrolateral preoptic nucleus (VLPO) also receives direct retinal input, in particular, from the retinal ganglion cells (Gooley et al., 2003) with melanopsin as the photopigment (Dacey et al., 2005). According to Saper and coworkers (2001), the VLPO is active during sleep and necessary for normal sleep. On the other hand, the posterior lateral hypothalamus contains orexin/hypocretin neurons that are crucial for maintaining normal wakefulness. The two regions have been suggested to provide a flip-flop switch that resists rapid and frequent transitions between sleep and waking (Saper et al., 2001). As orexin/hypocretin neurons interact with both sleep-active and wake-active neurons, Saper and colleagues hypothesized that orexin/hypocretin acts as stabilizer between wakefulness-maintaining and sleep-promoting systems, thus preventing sudden and inappropriate transitions between the two states. Interestingly, orexin/hypocretin neuronal activity is under the direct control of the SCN (Abrahamson et al., 2001) and peaks at the same time as the circadian alertness signal (Yoshida et al., 2001; Zeitzer et al., 2003). Furthermore, the VLPO also has indirect afferents via the

dorsomedial hypothalamus (DMH) from the SCN (Saper et al., 2005). It could be that an age-related reduction in the circadian input via the retinal ganglion cells and the DMH to the VLPO leads to more instability in the flip-flop switch and so to more involuntarily awakenings during sleep, and/or the influence of the SCN on orexin/hypocretin as a stabilizer for the flip-flop is reduced, particularly during the wake maintenance zone in the late evening and in the circadian sleep-promoting zone in the early morning hours. Indeed, there is recent evidence that orexin/hypocretin production in aged mice is altered such that the cell size of those neurons in the posterior and lateral hypothalamus is markedly reduced (M. Bentivoglio, personal communication).

Furthermore, new anatomical evidence for a circuit from the SCN via the DMH to major arousal-promoting cell groups (Deurveilher and Semba, 2005), including the locus coeruleus (LC), provides a basis for the circadian regulation of LC activity (Aston-Jones, 2005). The LC, in turn, influences the activity of a variety of central nervous system functions related to alertness, vigilance, and attention and also is related to thalamocortical oscillations, which modulate the throughput of sensory input to the cortex (Steriade, 2003). The important role of the SCN in these anatomical circuits, together with the age-related neuronal degeneration (Hofman and Swaab, 2005) in the SCN, emphasizes the possibility that the circadian timing system (process C) is crucially involved in age-related decrements of sleep consolidation.

In summary, our data favor the hypothesis that age-related changes in sleep structure, sleep consolidation, and sleep spindle characteristics are due to a reduction in the circadian force that opposes homeostatic pressure. This is particularly significant in the late evening, when the circadian drive for wakefulness is highest (*i.e.*, the wake maintenance zone) and in the early morning hours when the circadian drive for sleep is at its maximum. Therefore, along the lines of van Someren (2000), we suggest that manipulations of the circadian timing system, rather than the sleep homeostat, may offer a potential strategy to alleviate age-related changes in sleep and daytime alertness levels.

ACKNOWLEDGMENTS

We thank Claudia Renz, Giovanni Balestrieri, and Marie-France Dattler for their help in data acquisition; Drs. Carmen Schröder, Corina Schnitzler, Alexander Rösler and Tobias Müller for medical screenings; Kurt Kräuchi for statistical advice; and the volunteers for participating. This research was supported by Swiss National Foundation Grants START # 3130-054991.98 and #3100-055385.98 to CC, the Velux Foundation (Switzerland) and Bühlmann Laboratories, Allschwil (Switzerland).

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