Circadian and Homeostatic Sleep Regulation in Humans: Effects of Age and Monochromatic Light

Inauguraldissertation

zur
Erlangung der Würde eines Doktors der Philosophie
vorgelegt der
Philosophisch-Naturwissenschaftlichen Fakultät
der Universität Basel

von

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Basel, 2006

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät auf Antrag von

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Basel, den 4. April 2006

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SUMMARY

The first part of this thesis deals with age-related modifications in the circadian and homeostatic sleep regulation, whereas in the second part, the effects of an evening exposure to monochromatic light on subsequent sleep architecture and sleep electroencephalographic power spectra are described.

Age and sleep

Sleep in humans undergoes several age-related changes, resulting in less consolidated sleep, reduced slow wave sleep, advanced sleep-wake timing and shorter nocturnal sleep episodes. The first aim of this thesis was to gain comprehensive information about the influence of age on circadian and homeostatic aspects of sleep regulation. We compared the sleep electroencephalogram (EEG) of healthy young with older volunteers under high and low sleep pressure conditions. The study design consisted of two different protocols, both started with a baseline and ended up with a recovery night. The 40-h episode between these two nights comprised either an episode of total sleep deprivation (SD; high sleep pressure) or 10 sleep/wake cycles with 75 min of sleep followed by 150 min of wakefulness (low sleep pressure). The recovery nights served to investigate the age-related influence during enhanced and reduced sleep pressure conditions. The sleep episodes during the nap protocol allowed comparing circadian modulation of sleep characteristics between young and older subjects.

The response to high sleep pressure (i.e. after 40 hours of sleep deprivation) revealed a significantly attenuated frontal predominance of spectral EEG delta power in the sleep EEG of older participants, most pronounced at the beginning of the night (Chapter 2). In addition, the dissipation of homeostatic sleep pressure, as indexed by EEG delta power density, was shallower in the older than in the young group. This implies either an age-related weaker homeostatic response to sleep deprivation, predominantly in frontal brain areas, and/or altered cortical functions with an age-related higher vulnerability to sleep deprivation.

Under low sleep pressure (i.e. after multiple naps), older participants exhibited an attenuated occipital decline in delta frequencies in the all-night EEG during recovery sleep. This arose from an altered time course of EEG delta power density. The reduction of EEG delta activity after sleep satiation was similar in both age groups during the first sleep cycle. However, the EEG delta decrease to low sleep pressure was not longer present during the second sleep cycle in the older study group compared with the young (Chapter 4).

During the 40-h nap protocol (Chapter 3), we have quantitative evidence for a weaker circadian arousal signal in the older volunteers. This is reflected in higher subjective sleepiness levels during the late afternoon and evening ('wake maintenance zone'), with more sleep in the elderly during the naps at this time of day (Chapter 3). The day-night differences in the EEG lower alpha and spindle range were less pronounced in the older group. Furthermore, the amplitude of the circadian modulation of REM sleep was attenuated in the elderly and the nocturnal melatonin secretion was significantly reduced.

Taken together, our study revealed different responses to high and low sleep pressure, as assessed by the sleep EEG, subjective sleepiness levels and melatonin secretion, in older subjects when compared to the younger group. These results emphasize both the attenuation of circadian amplitude and alterations in homeostatic sleep regulation with age. We also gained insight into age-related differences in responsiveness of regional and time-dependent aspects of sleep. These age-related modifications are not uniformly spread over the brain and thus are likely to reflect differences in recovery or reactivation processes during sleep.

Light and sleep

Beside rods and cones, there is an additional so-called non-image-forming visual system (NIF) in the human retinal ganglion cells, with highest sensitivity in the 'blue' portion of visible light. The NIF is mediated by the photopigment melanopsin and projects to the circadian pacemaker, located in the suprachiasmatic nuclei (SCN). With efferents from the SCN to sleep- and wake-promoting brain regions, the NIF influences the circadian regulation of sleep and wakefulness. We compared sleep architecture and EEG spectra in young healthy men after evening exposure to

two different wavelengths of light (blue; 460 nm vs. green; 550 nm) or no light. The time course of EEG slow-wave activity (SWA; 0.75-4.5 Hz) after blue light was altered, with slightly lower SWA during the first and significantly higher SWA during the third sleep cycle in parietal and occipital brain regions. These findings could be interpreted either as the immediate induction of a circadian phase delay, or that the acute alerting effects of blue light continue into the sleep episode and are followed by an intra-sleep SWA rebound. Concomitantly, shorter REM sleep cycles after blue light exposure were observed during these two cycles. Our results show that the effects of light on human physiology including sleep not only depend on the duration and intensity of light but also on its wavelength, and thus further emphasize the critical role of the NIF in the regulation of sleep and circadian rhythms.

CHAPTER 1

GENERAL INTRODUCTION

Age in all its facets has become an important factor in our society, as the fraction of older persons (i.e. over 65 years) has more than doubled in the last hundred years and life expectancy is increasing (Bundesamt f. Statistik, 2004). Healthy ageing is known to cause various physiological and psychological modifications and among the most common are sleep problems, even though it is not well known what the underlying driving forces are. The first goal of this thesis was to analyse circadian and homeostatic influences of age on sleep-wake functions by means of the sleep EEG, subjective sleepiness and melatonin secretion.

The second topic of this thesis deals with the effects of monochromatic light on sleep in healthy young men, since it is known that several acute physiological responses to light are wavelength-dependent (e.g. melatonin suppression, sleepiness, heart rate, CBT). These findings are based on the recent discovery of a novel circadian photoreceptor in the mammalian retina, which provides the biological clockwork in the brain with non-visual light information.

Sleep and its analysis

The alternating succession of sleep (or rest) and wakefulness is a common feature in most invertebrates, vertebrates and also in humans (Campbell and Tobler, 1984; Tobler, 2005), although a comprehensive explanation of sleep function is still missing. In invertebrates and lower vertebrates (fish and amphibians), sleep is assessed by quiet resting behaviour, typical body position and reduced reactivity to external stimuli. In birds, reptiles and mammals, sleep and wakefulness are further ascertained in characteristically dynamic changes of electrical brain activity measured by the EEG. The EEG reflects summated electrical potentials of cortical neurons, registered from the surface of the scalp (i.e. EEG) or from the cortex

(electrocorticogram) in a voltage-time domain. The first EEG was described by Caton 1874 in dogs and apes, and by Berger 1929 in humans (Berger, 1929). Shortly afterwards, EEG differences between sleep and wakefulness were clearly recognised in humans (Loomis *et al.*, 1935, 1937).

Human sleep is not a uniform event but shows ultradian changes within each sleep episode (Dement and Kleitman, 1957). Each sleep cycle lasts about 90-100 min and normally comprises a non-rapid eye movement (NREM) and a rapid eye movement (REM) sleep episode. Visual scoring of the sleep EEG is defined according to Rechtschaffen and Kales (Rechtschaffen and Kales, 1968). In line with this standard, each NREM episode is characterised by a gradually lowering in frequency and a concomitant increase in amplitude of EEG waves from stage 1 (transition between wakefulness and sleep) to stage 3 and 4, (deep sleep). Stage 3 and 4 together are referred to as slow wave sleep (SWS). Additional phasic events such as sleep spindles and K-complexes (e.g. during stage 2) or vertex sharp transients (stage 1) are also typical incidents during NREM sleep. REM sleep, first described by Aserinsky and Kleitman (Aserinsky and Kleitman, 1953) is mainly characterised by rapid eye movements (measured in the electrooculogram; EOG), a loss of muscle tone in the electromyogram (EMG) and a low voltage, mixed EEG frequency pattern. In the course of the night, the percentage of SWS is highest at the beginning, whereas percentages of stage 2 and REM sleep increase during the second half of the night.

Based on arbitrary criteria (Rechtschaffen and Kales, 1968), visual scoring subdivides the sleep EEG into discrete units (stage 1 to 4, REM) and hence allows only limited quantification of continuous changes of the sleep EEG. The most common method to quantify the human EEG is by Fast Fourier transformation (FFT), which results in a power spectrum enabling analyses in the frequency domain (Dietsch, 1932; Borbély *et al.*, 1981). The FFT algorithm (Cooley and Tukey, 1965) transforms and integrates digitised EEG signals into sinusoid functions of varying frequency and amplitude per time window (e.g. 4-s epochs during sleep). Thus, the overall sleep EEG power density results in a 0.25 Hz resolution (μV^2/0.25 Hz), and the contribution of each 0.25-Hz frequency bin to the overall EEG power density during a certain time (e.g. across the night) can be assessed.

Sleep–wake regulation

The two process model

Two interacting processes, a homeostatic process S and a circadian process C have been postulated to regulate sleep and wakefulness (Figure 1; Borbély, 1982; Daan and Beersma, 1984).

Process S

The prior history of sleep and waking determines sleep propensity and sleep structure (Borbély *et al.*, 1981; Dijk *et al.*, 1993). With elapsed time awake, homeostatic sleep pressure increases during wakefulness and dissipates during the following sleep episode. Hallmarks of the homeostatic process are low frequency components such as the EEG theta activity (4.5-8 Hz) during wakefulness (Cajochen *et al.*, 1999b; Cajochen *et al.*, 2001; Finelli *et al.*, 2000) and the EEG slow-wave activity (SWA; 0.75-4.5 Hz) during sleep (Werth *et al.*, 1997; Cajochen *et al.*, 1999a; Finelli *et al.*, 2001). Process S has been shown to be operative in both animals and humans, and the predictions of the model, based on mathematical simulations, fit the experimental data (Achermann *et al.*, 1993). These responses reflect the homeostatic sleep regulatory process even though its function, neurobehavioral correlates and output signal(s) have not been elucidated so far. Restoration processes (Benington and Heller, 1995) of neuroactive substances as possible underlying mechanisms of homeostatic sleep regulation are discussed (Saper *et al.*, 2005b). There is growing evidence that also genetic (Franken *et al.*, 2001; Rétey *et al.*, 2005) and synaptic potentation functions (Tononi and Cirelli, 2003) are involved. The discussion in Chapter 2 deals also with local aspects of sleep and sleep function.

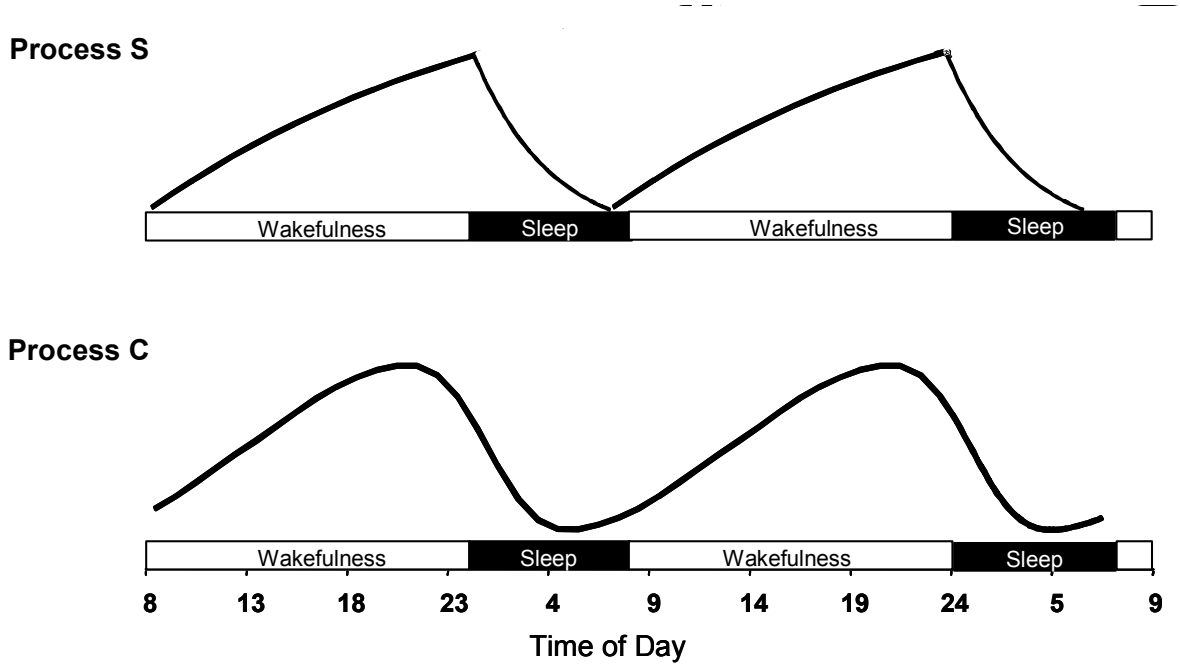

Figure 1

Schematic illustration of the two process model of sleep regulation in humans. The upper panel shows the homeostatic process S, where sleep pressure accumulates during wakefulness and declines during the sleep episode. The lower panel displays the circadian process C which influences sleep timing and propensity dependent on time of day. The dark areas depict the times where sleep normally occur (modified from Borbély, 1982).

Process C

Most physiological and behavioural variables in humans - such as heart rate, blood pressure, core body temperature (CBT), hormone levels, cognitive performance, subjective alertness and the sleep-wake rhythm undergo circadian rhythms with an approximately 24-h periodicity. Circadian rhythms are driven by an endogenous circadian pacemaker, located in the suprachiasmatic nuclei (SCN) of the hypothalamus (Moore and Eichler, 1972; Stephan and Zucker, 1972). The SCN is the master pacemaker in the mammalian brain which synchronises the circadian oscillators of most neuronal cells (Yamaguchi *et al.*, 2003) and peripheral tissues (heart, lung; for reviews see Reppert and Weaver, 2002; Schibler and Sassone-

Corsi, 2002). There is growing knowledge about the interaction of central and peripheral circadian oscillators in several tissues and cells, as well as the circadian regulation of (clock) gene expression in animals and humans (Reppert and Weaver, 2002; Schibler and Naef, 2005). Interestingly, most of these peripheral cells are also capable of oscillating independently of the SCN, e.g. after SCN lesions (Yoo *et al.*, 2004). The circadian rhythm mechanisms of different cell types are generated and sustained by transcription-translatory negative-feedback loops of clock genes (for reviews see Albrecht and Eichele, 2003). There is an explosion of research at the present time to better understand the cellular and molecular mechanisms of circadian rhythms underlying (human) physiology and pathophysiology (Stevens, 2005).

Besides food and social interactions (for a review see Mistlberger and Skene, 2005), light is the strongest zeitgeber for all species, synchronising the endogenous circadian clock to the 24-h day of the environment. Photobiotic activation is transmitted to the SCN via the retino-hypothalamic tract (see Figure 3 and Chapter 5). When external zeitgebers are absent, the endogenous circadian clock 'free-runs' with a period which is slightly different from 24 hours in humans (Aschoff and Wever, 1962; Czeisler *et al.*, 1999).

The circadian profile of melatonin secretion and CBT are reliable physiological hands of the clock and good markers of the circadian process in humans. Under entrained conditions, the onset of melatonin secretion occurs approximately 13 hours after habitual wake-up time, and CBT crests in the afternoon with a nadir approximately 2 hours before habitual wake time (Czeisler *et al.*, 1992; Duffy *et al.*, 1998). Sleep timing and structure are highly determined by circadian phase (Dijk and Czeisler, 1995; Dijk *et al.*, 1997). It has been shown that the circadian drive for sleep is highest in the early morning (around the CBT nadir), whereas the circadian drive for wakefulness is highest in the late evening, shortly before bedtime (Lavie, 1986; Strogatz *et al.*, 1987; Dijk and Czeisler, 1994). The paradoxical character of these two extremes of the circadian system can be explained by the interaction of homeostatic with circadian processes. In the course of a normal 16-h day, when homeostatic sleep pressure increases, a stronger wake-promoting signal is needed in the evening than in the morning, when sleep pressure is low, to counteract upcoming physiological and behavioural decrements. In contrast, throughout the night time sleep episode, when homeostatic sleep pressure dissipates, a circadian

sleep promoting signal is necessary to prevent premature awakening and to maintain sleep. This concept is drawn from studies with non-human primates and indicates that one function of the circadian system is to provide an alerting stimulus, which opposes the accumulating homeostatic sleep drive during waking hours (Edgar *et al.*, 1993). A recent positron emission tomography study in young subjects showed that evening wakefulness (in contrast to morning wakefulness) is associated with increased metabolism in brainstem and hypothalamic arousal systems, which could reflect the input from the circadian timing system to promote wakefulness and/or the effect of a high homeostatic sleep drive at the end of the waking day (Buysse *et al.*, 2004).

Besides the circadian there are also infradian (i.e. longer than 24 hours) and ultradian (i.e. less than 24 hours) processes, which oscillate in or out of phase with the endogenous circadian pacemaker and have additional modulatory influences on sleep-wake rhythms in humans.

Several kinds of experiments have served to quantify the circadian and homeostatic influences on sleep regulation. Since the early experiments in the Mammoth Caves in Kentucky, USA, 1938 (Kleitman, 1987) it has been shown that the lack of zeitgebers leads to free-running of the endogenous circadian clock in humans. In the famous bunker experiments under time-free isolation conditions, Aschoff described spontaneous internal desynchronization, where output markers of the endogenous clock (such as CBT) were free-running and oscillate at a stable period, whereas the (self-chosen) sleep-wake cycles occurred independently and were of instable lengths (Aschoff *et al.*, 1967; Aschoff and Wever, 1976). In those protocols, sleep was mostly initiated close to the CBT minimum. Additionally, the so called forced-internal desynchrony imposed artificial sleep-wake cycles (T-cycles) longer or shorter than 24 hours. As a consequence, both the length of the endogenous circadian period and the length of the scheduled sleep-wake cycles remained stable (Aschoff *et al.*, 1969). This experimental approach provided the basis for the so-called ‘forced desynchrony protocol’, where scheduled day-lengths are much shorter or longer than 24 hours (e.g. 21 or 28 hours) and therefore beyond the range of entrainment of the endogenous clock. Under these conditions, controlled for light, temperature and external time cues (Dijk and Czeisler, 1995), sleep and wakefulness are initiated at almost all different circadian phases. Thus, an evoked,

homeostatic element, which reflects the scheduled sleep-wake episodes, is separable from circadian dependent components (Dijk and Czeisler, 1995).

Another approach is the frequently used experimental manipulation of sleep pressure, for example in sleep deprivation protocols (SD; 'long days'). SD protocols allow quantification of the influence of homeostatic components on sleep regulation. The so-called constant routine (CR) protocol was further established to control for light, posture, time cues and food to assess circadian phase and amplitude under conditions with 'minimal masking' (Czeisler *et al.*, 1995). Further novel protocols under low sleep pressure ('short days' or nap protocols) serve to keep homeostatic sleep pressure on a relatively low level over the entire circadian cycle, and therefore allow the analysis of circadian processes on sleep and wakefulness in a complementary manner.

Neuronal aspects of sleep wake regulation

Sleep is assumed to be regulated by opposing wake- and sleep promoting-systems, as early described by von Economo (Von Economo, 1930). The integrated wake- and sleep-promoting information from the circadian timing system in the SCN of mammals is mediated via the ventral and dorsal subparaventricular zone (SPZ), the dorsomedial hypothalamic nucleus (DMH) and the medial preoptic area (Aston-Jones *et al.*, 2001; Mistlberger, 2005; Saper *et al.*, 2005b). The ascending reticular activating system (ARAS), first described from Moruzzi and Magoun (Moruzzi and Magoun, 1949), largely originates from a series of well-defined cell groups with identified neurotransmitters, such as the acetylcholine-producing cells in the laterodorsal tegmental and pedunculopontine nuclei, the noradrenergic nuclei of the locus coeruleus (LC), the serotonergic dorsal and median raphé nuclei (DR), the histaminergic tuberomammilary neurons (TMN) and the dopaminergic neurons in the periaequatorial grey matter (Saper *et al.*, 2001). These nuclei of the ARAS in the brainstem receive various inputs from visceral, somatic and sensory systems and project via two different pathways to the cerebral cortex (Jones, 2005). Excitatory neurons of the dorsal pathway in the ARAS project from the upper brainstem via relay and reticular neurons of the thalamus to the cortex and the ventral path

bypasses the thalamus and project directly to the cortex via the lateral hypothalamic area (LHT) and the basal forebrain (BF; Jones, 2005). The firing rate of glutaminergic ascending reticular neurons and monoaminergic cell groups as well as excitatory peptidergic neurons of the posterior hypothalamus and the LHT (which synthesises orexin) are crucial for maintaining cortical activation and behavioural arousal during wakefulness (Jones, 2005). The intralaminar and midline nuclei of the thalamus are also believed to play a role in cortical arousal (Saper *et al.*, 2005b). During sleep, gamma-aminobutrycacid (GABA)-containing neurons of the ventrolateral preoptic nucleus (VLPO) in the anterior hypothalamus inhibit neuronal activation of the ARAS, of the BF and of the cerebral cortex (Jones, 2005; Saper *et al.*, 2005b). Furthermore, GABA-ergic neurons in the reticular nucleus of the thalamus play an important role in generating sleep spindles and slow wave oscillations (Steriade *et al.*, 1993).

Saper et al. have proposed the so called 'flip-flop model of sleep and wakefulness' (Saper *et al.*, 2001). In this model, monoaminergic nuclei such as the TMN, LC and DR promote wakefulness by direct excitatory effects on the cortex and by inhibition of sleep promoting neurons of the VLPO. During sleep, the VLPO inhibits monoaminergic-mediated arousal regions through GABAergic and galaninergic projections (Saper *et al.*, 2001). Thus, intermediate states between sleep and wakefulness are prevented by the reciprocal inhibition of VLPO neurons and monoaminergic cell groups which concomitantly disinhibit and reinforce their own firing rates. Orexin-containing neurons seem to play an important stabilising role in the proposed flip-flop mechanism.

Age-related changes in sleep and wakefulness

Healthy ageing encompasses a number of systematic changes in physiological and neurobehavioral functions, whereby sleep problems, increased sleepiness during the daytime with naps, and reduced cognitive performance are a common problem (Miles and Dement, 1980; Bliwise, 1993; Prinz, 2004). The hallmarks during night sleep are less consolidated sleep episodes with involuntarily awakenings, altered sleep timing and sleep structure (Bliwise, 1993; Prinz, 2004). This suggests an age-related disruption of sleep, which starts already in the middle years of the human lifespan (Carrier *et al.*, 2001). Whether circadian or homeostatic factors, or both, contribute to

this deterioration in sleep regulation with age, is still not clear. It has also been stated that that ageing *per se* does not cause sleep disruption, but rather the ability to sleep decreases with age (Ancoli-Israel and Cooke, 2005), even though healthy older people do not have primarily more problems to initiate sleep. During night time they awake more frequently but fall back asleep at the same rate as the young (Klerman *et al.*, 2004).

It is well documented that the total amount of SWS and SWA decreases with age (Miles and Dement, 1980; Buysse *et al.*, 1992; Prinz, 1995; Landolt *et al.*, 1996; Bliwise, 2000), a process which starts during adolescence (Gaudreau *et al.*, 2001a). The age-related reduction in SWS occurs at all circadian phases (Dijk *et al.*, 1999a). The function of this decrease and its repercussion on the homeostatic sleep regulation are not known (Figure 2).

There are also gender differences, such that independent of age, women show higher levels of absolute SWS and SWA under baseline conditions (Dijk and Beersma, 1989; Carrier *et al.*, 2001). Whether these gender differences are related to differences in skull thickness, as originally proposed (Dijk and Beersma, 1989), or are more deeply physiological, is not yet clear.

Predominantly circadian related changes in the sleep-wake cycle with age have been reported. Older individuals usually show advanced bed- and wake times of approximately one hour when compared to the young (Duffy *et al.*, 1998; Dijk *et al.*, 2000; Duffy *et al.*, 2002), but this does not always appear in combination with advanced circadian phase or alterations in phase angle (Carrier *et al.*, 1999; Kripke *et al.*, 2005; Monk, 2005). A possible explanation for the age-related circadian phase advance could be the exposure to more morning light in the elderly due to frequent early awakenings, since light in the morning has a phase advancing effect in humans (Honma *et al.*, 1987; Czeisler *et al.*, 1989; Minors *et al.*, 1991; Khalsa *et al.*, 2003). Thus, it is possible that zeitgeber strength in the morning would increase with age and reinforce a phase advance every day. However, this hypothesis has been rejected by studies under constant dim light conditions (in forced desynchrony or constant routine protocols) where older subjects still remain phase advanced when compared to the young study group (Dijk and Duffy, 1999; Dijk *et al.*, 1999a). A recent study in middle-aged subjects clearly showed that the age-related advance of the circadian melatonin phase could not be entirely explained by the change in

habitual light exposure and corresponding shifts in the dim light melatonin onset (DLMO; Kawinska *et al.*, 2005).

Furthermore, phase response curves to bright light pulses at different times of day in the young and elderly reveal a similar magnitude of phase delays in both groups. Interestingly, the induction of phase advances was significantly attenuated in older people (Klerman *et al.*, 2001).

The circadian amplitude of phase markers such as melatonin and CBT and cortisol declines markedly with age (Weitzman *et al.*, 1982; Van Coevorden *et al.*, 1991; Czeisler *et al.*, 1992), even though these findings are far from being consistent in

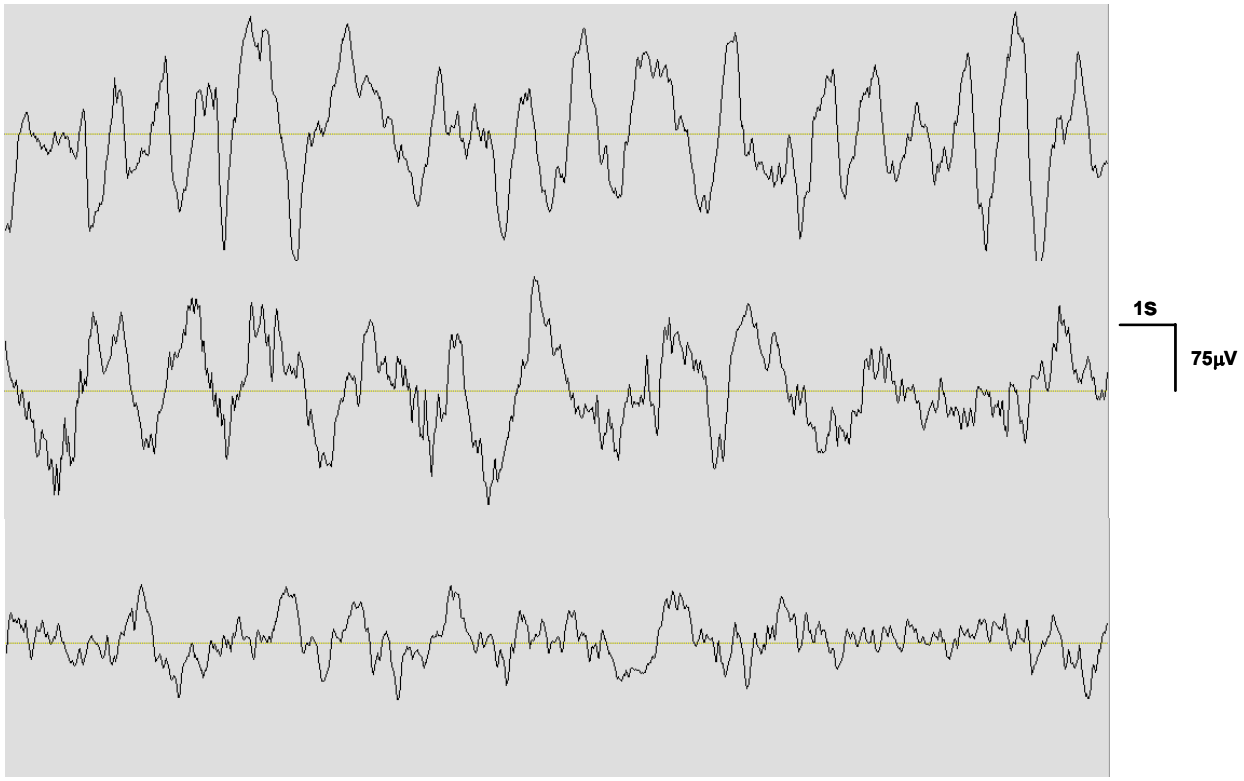

Figure 2

Figure 2 shows the qualitative age differences in the absolute EEG delta (µV) activity from a central derivation (C3) across a 20s epoch between one young 21 year old man with well preserved EEG delta power (upper panel), and two elderly: 61 year old women and 69 year old men (middle and lower panel). Although age differences are clear, there are also obviously marked inter-individual differences between the two older subjects (middle and lower panel).

the literature (Zeitzer *et al.*, 1999; Niggemyer *et al.*, 2004; Monk, 2005). A possible explanation for those inconsistencies might be the evidence of great(er) inter-individual differences, different protocols, and masking factors such as sleep or body position. In the sleep EEG, the circadian amplitude of EEG power density in the sleep spindle range is attenuated with age (Wei *et al.*, 1999). Some studies have also reported a decrease in REM sleep amplitude (Haimov and Lavie, 1997). Hence, it is not clear if the age-related circadian impairments are due to less functionality of the circadian pacemaker with subsequently decreased output signals, as it may be for the decreased amplitude in melatonin secretion; and/or if this weakening in SCN function leads to less robustness, i.e. a higher vulnerability to misalignments in circadian timing.

On the other hand, the endogenous period in humans (tau) is not affected with age and remains stable at about 24.18h (Czeisler *et al.*, 1999). Thus, the age-related circadian phase advance in humans cannot be explained by a shortening of endogenous period.

Long and short sleepers and patients suffering from delayed or advanced sleep phase syndrome do not show the same phase angle differences as young and older subjects (Dijk and Lockley, 2002; Aeschbach *et al.* 2003).

Taken together, several age-related deteriorations in sleep have been reported, even though the causal context of contributing factors remains to be elucidated, in particular, the quantified interaction of homeostatic with circadian processes in healthy aged persons. One possible explanation for the decrease in circadian amplitude of physiological variables in healthy elderly might be related to age-related visual impairments. It is known that retinal pathways are impaired by several age-related degeneration processes (Marshall, 1985; Young, 1987). Changes in lens properties (‘yellowing the lens’; Charman, 2003) start already in the fourth decade of life. Moreover, the elderly suffer more often from cataracts, macula degeneration or glaucoma, all of which impairs the input of light to the retino-hypothalamic tract. In patients with cataracts, sleep complaints, sleepiness in the morning and daytime sleepiness 9 months after cataract surgery had all decreased (Asplund and Lindblad, 2004). Therefore, the recent discovery of a new, non-image-forming circadian photorecepter (see next paragraph and Chapter 5), raises the

possibility of finding new ways to treat age-dependent circadian aspects of sleep regulation.

Influence of monochromatic light on human physiology

The response of the circadian pacemaker to ocular light exposure varies with both the timing and the light intensity of photic stimuli (Brainard *et al.*, 1988; Roenneberg and Foster, 1997; Czeisler and Wright, 1999).

The timing of light exposure induces different circadian phase shifts in humans, which has been assessed by human phase response curves; with an advancing portion after light exposure in the morning (i.e. light after the CBT minimum) and a delaying portion after light in the evening and night, i.e. before the CBT minimum (Honma *et al.*, 1987; Czeisler *et al.*, 1989; Minors *et al.*, 1991; Khalsa *et al.*, 2003). Polychromatic light acts also acutely on human physiology, eliciting melatonin suppression (Lewy *et al.*, 1980), increasing CBT (Badia *et al.*, 1991; Dijk *et al.*, 1991; Cajochen *et al.*, 1992), heart rate (Scheer *et al.*, 2004) and alertness (Badia *et al.*, 1991; Cajochen *et al.*, 2000) and cortisol (Scheer and Buijs, 1999; Leproult *et al.*, 2001).

The effects of different polychromatic light intensities (lux) on the circadian timing system in humans have been demonstrated with dose-response curves of single light episodes during the phase delaying portion of the night (Boivin *et al.*, 1996; Zeitzer *et al.*, 2000). The results are characterised by a logistic regression function with high sensitivity to light, such that after evening exposure to only 1% (≈100 lux, dim room light) of the portion of bright light (≈9100 lux), half of the maximal phase-delaying response was obtained (Zeitzer *et al.*, 2000). Similar responses were achieved in melatonin suppression and both subjective and objective measures of alertness (Cajochen *et al.*, 2000; Zeitzer *et al.*, 2000).

Not only the timing and intensity of light, but also its wavelength(s) play a crucial role for the circadian timing system in mammals. The mammalian circadian timing system seems most sensitive to shorter wavelengths of visible light as has been shown with irradiance-response curves on melatonin suppression (Brainard *et al.*, 2001b; Thapan *et al.*, 2001). Beside rods and cones there is a third photoreceptor in the retinal ganglion cells (RGC), which is responsible for the so-called non-image-

forming system in mammals (for a review see Foster, 2005), capable of driving the circadian rhythm of light via the retino-hypothalamic tract to the SCN and to the periphery (Berson *et al.*, 2002; Hattar *et al.*, 2002; Provencio *et al.*, 2002). The biological activity of the new photoreceptor peaks in humans between 446 and 477 nm, which is in the blue portion of the visible light spectrum (Brainard *et al.*, 2001b; Thapan *et al.*, 2001). Furthermore, the photopigment melanopsin was discovered as the responsible ligand molecule with a wide-spread action spectrum throughout the brain (Dacey *et al.*, 2005; Panda *et al.*, 2005; Qiu *et al.*, 2005).

The known efferents of melanopsin-containing cells in the retino-hypothalamic tract, and their influence on human physiology and sleep/wake cycles are schematically illustrated in Figure 3. The RGCs project to neurons in the SCN, the pretectal area (PTA), the intergeniculate leaflet (IGL) and also the ventral subparaventricular zone (vSPZ; which also receives inputs from the IGL) and to the VLPO (Gooley *et al.*, 2003). The vSPZ, and to a lesser extent the SCN, directly project via melanopsin containing neurons to the VLPO (Chou *et al.*, 2002). There are also melanopsin-containing projections between the SCN and the IGL. On the other hand, neuronal activity of the SCN appears to be also strongly and differentially influenced by alternations between sleep states. It has been shown in rodents that SCN neurons change their circadian firing pattern parallel with the sleep-wake cycle (Deboer *et al.*, 2003).

The physiological responses induced by visible light become more effective when monochromatic light in the blue portion of the spectrum is used, compared with monochromatic light of longer wavelengths (e.g. green, red). These physiological responses comprise melatonin suppression, circadian phase shifts, increases in CBT, heart rate, alertness, performance and changes in the waking EEG, as well as changes in pupil width and the human electroretinogram (Brainard *et al.*, 2001a; Thapan *et al.*, 2001; Hankins and Lucas, 2002; Lockley *et al.*, 2003; Warman *et al.*, 2003; Cajochen, 2005; Lockley *et al.*, 2006). Clock gene expression of *PER2* was also significantly higher after blue light at 460 nm when compared to green light at 550 nm (Cajochen *et al.*, 2006a). Despite the knowledge of anatomical connections via the RGCs to the SCN, and direct and indirect pathways to sleep- and wake-promoting brain areas, it is not yet elucidated if and how sleep architecture and sleep

EEG spectra are changed by low intensity monochromatic light exposure in the evening.

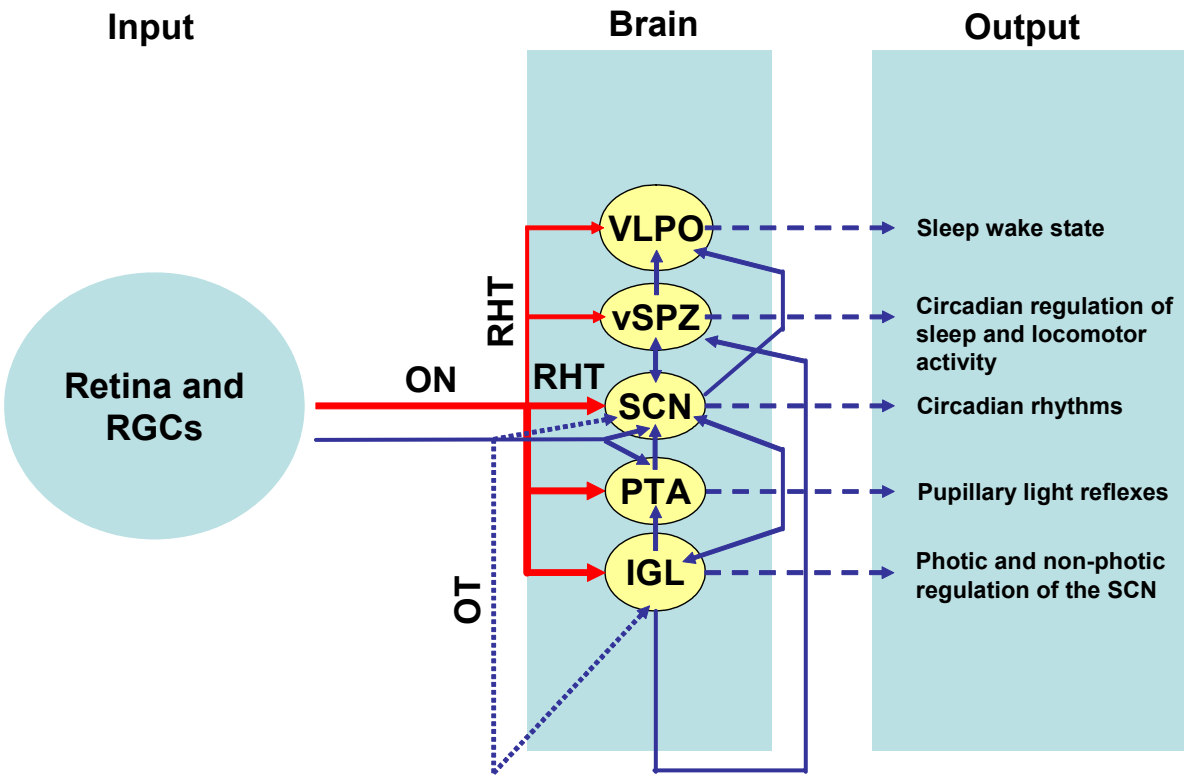

Figure 3

Schematic illustration showing the pathway of the RGCs in the retina which express the photopigment melanopsin. The solid arrows indicate projections from the RGCs to the SCN, the PTA, the IGL, the vSPZ and the VLPO via ON. The thickness of these arrows reflects approximately the density of the projections. Solid lines from the RGC's show collaterals to the SCN and the PTA as well as melanopsin containing pathways between IGL, PTA, SCN, vSPZ and VLPO. Dotted lines indicate proposed axons collaterals to the SCN and the IGL. Dashed arrows depict the physiologic and behavioural output of the melanopsin conducted actions. **RGCs**: retinal ganglion cells; **ON**: optic nerve; **OT**: optic tract; **RHT**: retino-hypothalamic tract; **IGL**: intergeniculate leaflet; **PTA**: pretectal area; **SCN**: suprachiasmatic nuclei; **vSPZ**: ventral subparaventricular zone; **VLPO**: ventrolateral preoptic nucleus (Redrawn from Gooley et al. 2003)

Objectives and methods for the thesis

The general objectives of this thesis can be segregated into two parts: The first part (Chapters 2-4) aimed to further extend the knowledge of age-related decrements in sleep regulation. The main purpose was to quantify the contribution of the homeostatic and the circadian process on sleep regulation in healthy young and older volunteers. The homeostatic influence was assessed by means of the sleep EEG along the midline derivations, and the circadian influence additionally encompassed analyses of melatonin and subjective sleepiness scales. The study consisted of two different protocols which were applied in a balanced and gender-matched cross-over design, in the distance of 1-3 weeks between the study blocks (Figure 4). Both protocols started with a baseline night, followed either by 40 hours of wakefulness (high sleep pressure; Figure 4, left panel) or by a 40-h multiple nap protocol with scheduled 75/150min sleep-wake episodes (low sleep pressure; Figure 4, right panel). Both protocols ended with a recovery night.

The second chapter deals with the different influence of high sleep pressure (after SD) on NREM sleep during the recovery night in the young and elderly, with focus on topographical distribution of EEG power density; whereas in the fourth chapter the homeostatic influence on sleep under low sleep pressure (after intermittent napping) is reported. The third chapter targets age-related changes in the circadian regulation of sleep and wakefulness during the multiple 40-h nap protocol.

The objective of the second part of this thesis (Chapter 5) was the assessment of the influence of different wavelengths of evening exposure of monochromatic light on sleep architecture and spectral components of the sleep EEG in young healthy men. The protocol comprised a balanced crossover design with two different 2-h light exposures at low intensities and one no-light control condition (see Chapter 5, Figure 1). Before evening light and no-light exposures, the subjects underwent 2 hours of dark adaptation. After light and no-light exposure the subjects were allowed to sleep.

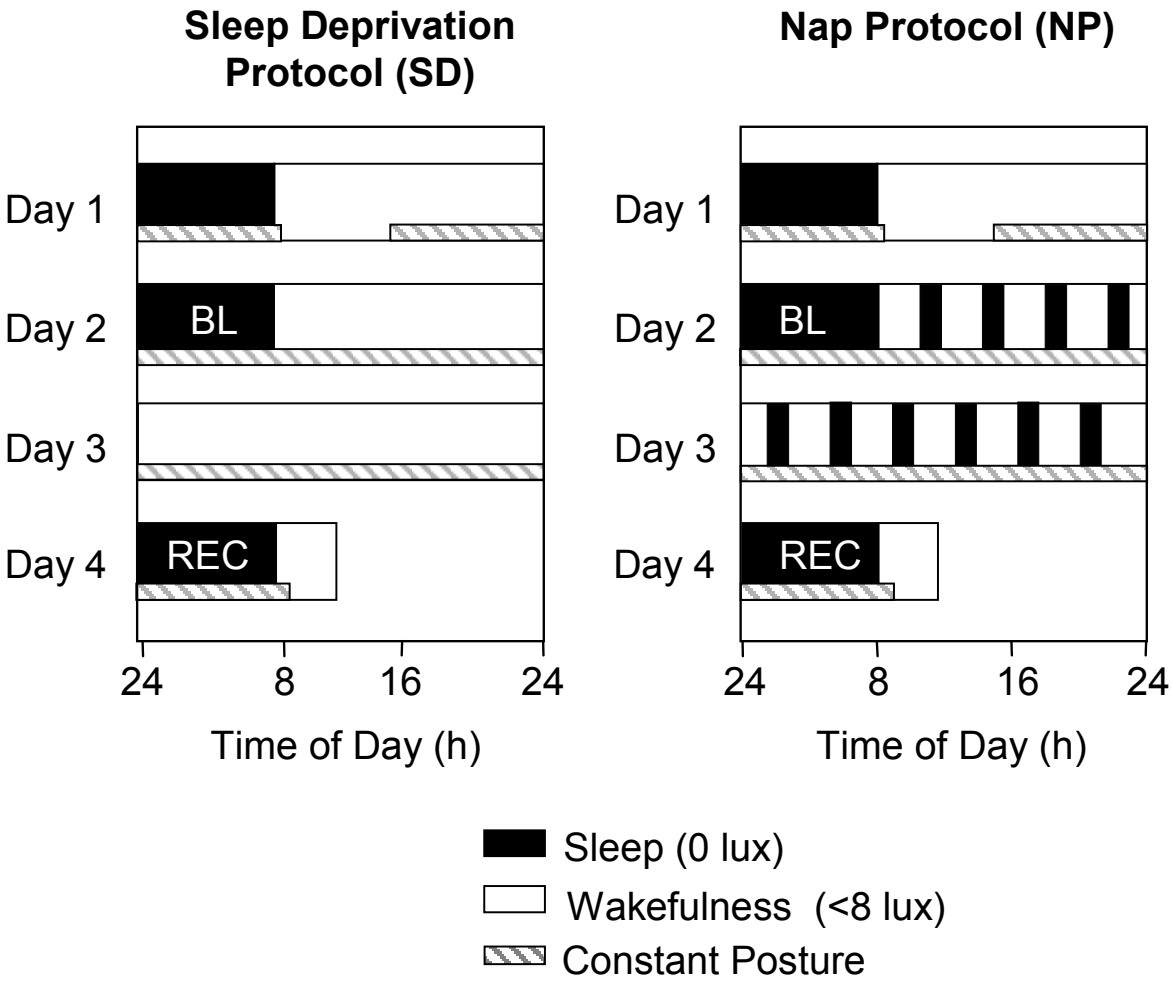

Figure 4

Schematic representation of the two protocols for high (left side) and low sleep pressure (right side). White areas indicate scheduled wakefulness and dark bars delineate the scheduled sleep episodes. Hatched bars depict constant posture, semi-recumbent during wakefulness and recumbent during sleep. BL=baseline night, REC=recovery night

CHAPTER 2

THE FRONTAL PREDOMINANCE IN HUMAN EEG DELTA ACTIVITY AFTER SLEEP LOSS DECREASES WITH AGE

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Published in: European Journal of Neuroscience (2004), 20: 1402-1410

Summary

Sleep loss has marked and selective effects on brain wave activity during subsequent recovery sleep. The electroencephalogram (EEG) responds to sleep deprivation with a relative increase in power density in the delta and theta range during non-rapid eye movement sleep. We investigated age-related changes of the EEG response to sleep deprivation along the antero-posterior axis (Fz, Cz, Pz, Oz) under constant routine conditions. Both healthy young (20-31 years) and older (57-74 years) participants manifested a significant relative increase in EEG power density in the delta and theta range after 40 hours of sleep deprivation, indicating a sustained capacity of the sleep homeostat to respond to sleep loss in ageing. However, the increase in relative EEG delta activity (1.25-3.75 Hz) following sleep deprivation was significantly more pronounced in frontal than parietal brain regions in the young, whereas such a frontal predominance was diminished in the older volunteers. This age-related decrease of frontal delta predominance was most distinct at the beginning of the recovery sleep episode. Furthermore, the dissipation of homeostatic sleep pressure during the recovery night, as indexed by EEG delta activity, exhibited a significantly shallower decline in the older group. Activation of sleep regulatory processes in frontal brain areas by an extension of wakefulness from 16 to 40 hours appears to be age-dependent. These findings provide quantitative evidence for the hypothesis that frontal brain regions are particularly vulnerable to the effects of elevated sleep pressure (‘prefrontal tiredness’) and ageing (‘frontal ageing’).

Introduction

According to the two-process model of sleep regulation, sleep pressure accumulates during wakefulness and dissipates in the course of the following sleep episode (Borbély, 1982). EEG activity in low frequency components (0.75-7.0 Hz) is the key electrophysiological marker of this homeostatic process (Borbély *et al.*, 1981) - most apparent in frontal brain areas during sustained wakefulness (Cajochen *et al.*, 1999b; Cajochen *et al.*, 2001; Finelli *et al.*, 2001) and during the following sleep episode (Werth *et al.*, 1997; Cajochen *et al.*, 1999a; Finelli *et al.*, 2000). Positron emission tomography (PET) studies have demonstrated that the decline of regional cerebral blood flow (rCBF) during slow wave sleep (SWS) is most prominent in frontal cortical areas (Maquet *et al.*, 1990; Braun *et al.*, 1997; Hofle *et al.*, 1997; Maquet *et al.*, 1997; Kajimura *et al.*, 1999; Nofzinger *et al.*, 2002). Thus, frontal brain areas, especially the prefrontal cortex (PFC) may represent a brain region particularly vulnerable to the effects of sleep loss (Horne, 1992; Horne, 1993; Harrison *et al.*, 2000; Thomas *et al.*, 2000; Jones and Harrison, 2001; Muzur *et al.*, 2002).

Besides sleep regulatory processes, there is mounting evidence that the PFC is also susceptible to age-related changes (Moscovitch and Winocur, 1995; Gunning-Dixon and Raz, 2003; Tisserand and Jolles, 2003). Neurobehavioral functions highly dependent on prefrontal cortical regions decline with age, whereas those less dependent on the PFC remain better conserved (e.g. Dempster, 1992; for a critical review see Greenwood, 2000).

Both the general enhancement of recuperative SWA-response to sleep loss in the young and age-related changes in neurobehavioral functions are associated with the PFC. How these two processes modify the sleep EEG along the antero-posterior axis has, to our knowledge, not yet been investigated. Older volunteers exhibit lower absolute levels of slow-wave activity (SWA; 0.75-4.5 Hz) during sleep episodes, as a result of a progressive decline starting already in their second decade of life (Smith *et al.*, 1977; Ehlers and Kupfer, 1989; Landolt *et al.*, 1996; Carrier *et al.*, 2001; Landolt and Borbély, 2001). There is also evidence that middle-aged volunteers exhibit a longer time constant in the dissipation of SWA during baseline nights (Dijk *et al.*, 1989b). However, these data are largely based on a single derivation from central brain regions. Therefore, we aimed at quantifying the homeostatic response to sleep

deprivation in the spectral composition of the sleep EEG along the antero-posterior brain axis, comparing healthy young with older participants. If functions subserved by the PFC are impaired in non-pathological ageing, and if age modifies homeostatic sleep regulation after sleep deprivation, two compensatory reactions of the PFC are possible: an increase of PFC activity (the PFC needs to be more active to sustain high neurobehavioral performance) or a decrease (the PFC cannot adequately compensate for the augmented duration of wakefulness). Based on the aforementioned literature we predicted the latter and hypothesised that sleep deprivation will lead to a less pronounced increase in frontal low EEG components during the recovery night. Furthermore, we predicted a shallower SWA decline during the recovery sleep episode in older compared with young volunteers. To test these hypotheses, we examined both age groups during a 40-h sleep deprivation protocol under stringently controlled constant routine conditions.

Methods

Study participants

Study volunteers were recruited via advertisements at different universities and in newspapers in Switzerland. Sixteen young (8 women and 8 men, age range 20-31 years, mean: 25±0.9 SEM) and sixteen older volunteers (8 women and 8 men, age range 57-74 years, mean: 64.9±1.4) were included following initial screening of more than 500 potential applicants. All study participants were non-smokers, did not take any drugs (urinary drug screening before study begin) or medication, and were free from medical, psychiatric and sleep disorders. Four young female volunteers used oral contraceptives. All young women were studied during the follicular phase of their menstrual cycle. The health of all volunteers was assessed by questionnaires, physical examination, interviews and a polysomnographically recorded screening night. During the baseline week preceding the study, volunteers were instructed to keep their individual bed- and wake-time within a self-selected range of ± 30 minutes, and to attempt to sleep for eight hours. This was assessed by a wrist activity monitor (Cambridge Neurotechnologies®, Cambridge, UK) and sleep logs. Study participants were asked to abstain from excessive caffeine and alcohol consumption. The study

protocol, the screening questionnaires and the consent form were approved by the Ethical Committee of Basel, Switzerland, and were in agreement with the Declaration of Helsinki. After a thorough personal discussion of all protocol details with an investigator, the study participants gave their written informed consent.

Protocol

The protocol consisted of two baseline nights in the sleep laboratory followed by a 40-h episode of sleep deprivation and an 8-h recovery sleep episode. The entire protocol was carried out under constant routine (CR) conditions (<8 lux, temperature 21°C, semi-recumbent posture in bed, regular small isocaloric snacks and water, and no time cues, Czeisler *et al.*, 1985; for details see Cajochen *et al.*, 2001). The timing of the 8-h sleep episodes during the laboratory study was scheduled by centring the midpoint of the study participants' habitual sleep episodes at home during the baseline week (as assessed by actigraphy). Continuous polysomnographic recording started after the first baseline night. The older study participants received a daily low-dose heparin injection (Fragmin® 0.2ml, 2500 IE/UI, Pharmacia AG, Dübendorf, Switzerland) while recumbent in the CR.

Sleep EEG recordings and analysis

The sleep EEG was recorded from twelve derivations (F3, F4, Fz, C3, C4, Cz, P3, P4, Pz, O1, O2, Oz) referenced against linked mastoids (A1, A2), together with two electrooculograms (EOG), one electrocardiogram (ECG) and one submental electromyogram (EMG) using a digital ambulatory sleep recording system (Vitaport-3 digital recorder, TEMEC Instruments BV, Kerkrade, The Netherlands). All signals were filtered at 30 Hz (4th order Bessel type anti-aliasing low-pass filter, total 24dB/Oct). A time constant of 1.0 s was used prior to on-line digitisation (range 610 μV, 12 bit AD converter, 0.15 μV/bit, sampling rate at 128 Hz for the EEG). The raw signals were stored on a Flash RAM card (Viking, USA) and downloaded off-line to a local computer hard drive. Sleep stages were visually scored per 20-s epoch according to standard criteria (Rechtschaffen and Kales, 1968). Artefact-free sleep

EEGs (automated artefact detection algorithm: CASA, 2000 PhyVision BV, Gemert, The Netherlands) were subjected to spectral analysis using a Fast-Fourier-Transformation (FFT, 10% cosine 4-s window) resulting in a 0.25-Hz bin resolution. For data reduction, artefact free 4-s epochs were averaged over 20-s epochs. Sleep EEG power spectra were calculated during NREM sleep (stages: two, three and four) in the frequency range from 0.5 to 32 Hz. Here, we report EEG power density derived from the midline (Fz, Cz, Pz and Oz) during NREM sleep in the range from 0.75 to 25 Hz.

Statistics

The statistical packages SAS® (SAS Institute Inc., Cary, NC, USA; Version 6.12) and Statistica® (Stat Soft Inc., 2000. STATISTICA for Windows, Tulsa, OK, USA) were used. Two-, three-, and four-way analyses of variance for repeated measures (rANOVA) with the factors 'age' (young vs. older), 'derivation' (Fz, Cz, Pz and Oz) and 'night' (baseline, recovery) or 'time interval' (2-h intervals) were performed for each EEG power value in each frequency bin separately. Analyses were based on log-transformed EEG power density (μV^2/0.25 Hz) and on relative EEG power density (log-ratios, %). Prior to plotting, the data were averaged across subjects, then re-transformed and expressed as a percentage of the baseline night values. All *p*-values derived from rANOVAs were based on Huynh-Feld's (H-F) corrected degrees of freedom, but the original degrees of freedom are reported. *Post-hoc* comparisons were performed by using Duncan's multiple range test (corrected for multiple comparisons; $p<0.05$ was considered significant). For two *post-hoc* comparisons in Figure 3 non-parametric tests (Mann-Whitney U test and Wilcoxon matced-pairs test) were applied, since the values in the older group did not fulfil criteria for normal distribution.

Results

Sleep measures derived from visual scoring during the baseline and the recovery night

Table 1 summarises sleep measures during the baseline and the recovery night (% of total sleep time). Two-way rANOVAs with the factors 'age' and 'night' were performed for each variable separately. A main effect of age was found for the variables: total sleep time (TST), sleep efficiency (SE), wakefulness after sleep onset, arousal after sleep onset, stage two, stage four, SWS, NREM sleep, REM sleep (for all measures: $F_{1,30}>5.5$; $p<0.05$) and a tendency for movement time (MT) and stage one ($p<0.1$). The main factor 'night' yielded significance in all variables (for all measures: $F_{1,1}>6$; $p<0.05$) except for MT, NREM sleep, REM sleep and REM latency. The interaction between the factors 'age' x 'night' yielded significance for stage four, SWS as well as sleep latencies to stage one and two ($F_{1,30}>4$; $p<0.05$). *Post-hoc* comparisons revealed that the older volunteers had significant less SWS during both the baseline and the recovery night (Duncan's multiple range test; $p<0.05$). *Post-hoc* comparisons performed for sleep latency one and two indicated significant shorter sleep latencies during the recovery night for the young group (Duncan's multiple range test; $p<0.05$; sleep latencies were calculated on log-transformed values).

Table 1

Relative values (percent of total sleep time) are shown for sleep stages

	Baseline Night		**Recovery Night**				
	Young	**Older**	**Young**	**Older**	Age	Night	Age x Night
TST(min)	438.1±7.2	407.0±8.4	449.9±9.8	429.1±7.2	*	*	
SE (%)	91.3±1.5	84.8±1.7	93.9±2.1	89.5±1.5	*	*	
% MT	0.6±0.5	0.2±0.3	1.6±0.4	0.3±0.6	°		
% Wakefulness	4.5±1.5	14.5±2.4	1.3±0.5	8.0±1.6	**	*	
% Arousal	7.6±1.7	16.7±2.5	3.8±0.8	10.3±1.8	**	*	
% Stage 1	12.6±1.4	14.6±1.6	6.5±0.8	9.7±0.8	°	**	
% Stage 2	50.3±1.3	59.6±2.7	46.5±1.2	57.6±3.1	*	*	
% Stage 3	10.3±0.7	8.0±1.4	13.7±1.2	12.7±1.2		**	
% Stage 4	6.9±1.5	1.7±0.5	14.3±2.0	4.6±1.1	**	**	*
% SWS	17.2±1.7	9.7±1.9	27.9±1.6	17.3±2.3	*	**	*
% NREM	80.0±1.0	83.9±1.3	80.9±1.2	84.5±1.4	*		
% REM	20.0±1.0	16.1±1.3	19.1±1.2	15.5±1.4	*		
SL1 (min)	10.2±2.3	8.0±0.8	3.9±0.6	8.2±1.8		**	*
SL2 (min)	15.2±2.3	11.0±0.9	6.3±0.8	10.4±1.8		**	**
RL (min)	78.9±5.9	92.5±21.4	73.6±8.7	71.1±7.3			

TST=total sleep time (min; stages 1-4 + REM sleep); SE=(TST/time after lights off) x 100; % MT=movement time (after sleep onset) x 100; % wakefulness (after sleep onset) x 100; % arousal=(wakefulness + movement time) x 100, (after sleep onset); % SWS=(stage 3 + stage 4) x 100; % NREM sleep=(SWS + stage 1 + stage 2) x 100; SL1=sleep latency to stage 1 (min); SL2=sleep latency to stage 2 (min); RL=REM latency to REM sleep (min); *=$p<0.05$, **=$p<0.001$, °=$p<0.1$. Values are indicated ± 1 SEM (n=16 in each age group).

Age-related changes in EEG power density (0.75-25 Hz) during the baseline and the recovery night after sleep loss

To examine EEG power density in the range of 0.75-25 Hz during NREM sleep for both age groups, all-night power density during the recovery and the baseline night were calculated for the midline derivations (Fz, Cz, Pz and Oz) in each frequency bin (Figure 1). A three-way rANOVA (on log-transformed values) with the factors 'age', 'derivation' and 'night' yielded a main effect for the factor 'night' in all frequency bins (p<0.05) except within the higher spindle frequency range from 14.5-15.75 Hz. A main effect of 'age' was found in the frequency bins 0.75-5.25 Hz and 6.75-7.75 Hz (delta and theta range) and 11.75-14.75 Hz (spindle range). Figure 1 shows higher absolute EEG power in those frequency bins for the young than for the older age group. The interaction of the factors 'age' x 'night' and 'age' x 'derivation' respectively was significant for some frequency bins in the delta as well as in the spindle frequency range (p<0.05 for each frequency bin, bottom panel in figure 1). The interaction for these three factors was significant in the frequency bins between 1.25 and 3.75 Hz.

For analysing the overall response to elevated sleep pressure during the recovery night in the young and older study participants, the absolute EEG power spectra were calculated for each derivation and frequency bin and expressed as percentage of the baseline values (Figure 2). A two-way rANOVA (log-ratios) with the factors 'age' x 'derivation' yielded a significant interaction in the frequency bins between 1.25 and 3.75 Hz (p<0.05 for each frequency bin; vertical dotted lines in Figure 2 emphasise the significant frequency range). Further analyses with both age groups were based on a collapsed EEG delta band in the range of 1.25-3.75 Hz, since these frequency bins exhibited a significant 3-way interaction.

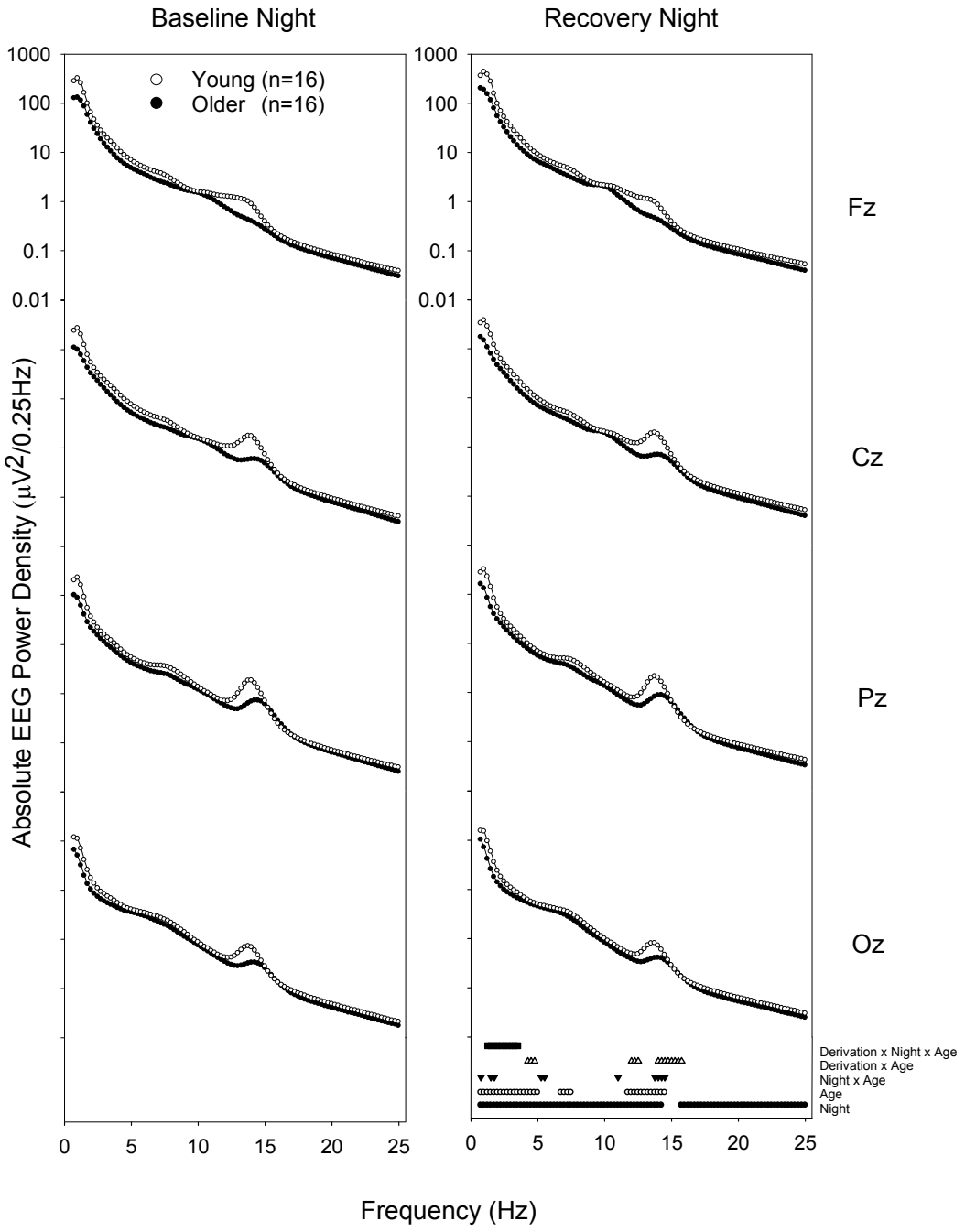

Figure 1

Absolute EEG power spectra during NREM sleep in the midline derivations (Fz, Cz, Pz and Oz) during the baseline (left panel) and the recovery night (right panel) for the young (=open circles) and the older age group (=filled circles). Mean values are shown for each 0.25 Hz frequency bin in the range of 0.75-25 Hz (n=16 in both age groups). Horizontal circles near the abscissa at the bottom indicate frequency bins for which the factor 'night' (filled circles) and the factor 'age' (open circles) were significant. Filled black triangles show frequency bins for which the interaction 'age' x 'night' turned out to be significant and white triangles represent the significant values of the interaction for the factors 'age' x 'derivation'. Black squares at the bottom indicate frequency bins for which the interaction 'age' x 'night' x 'derivation' yielded significant values.

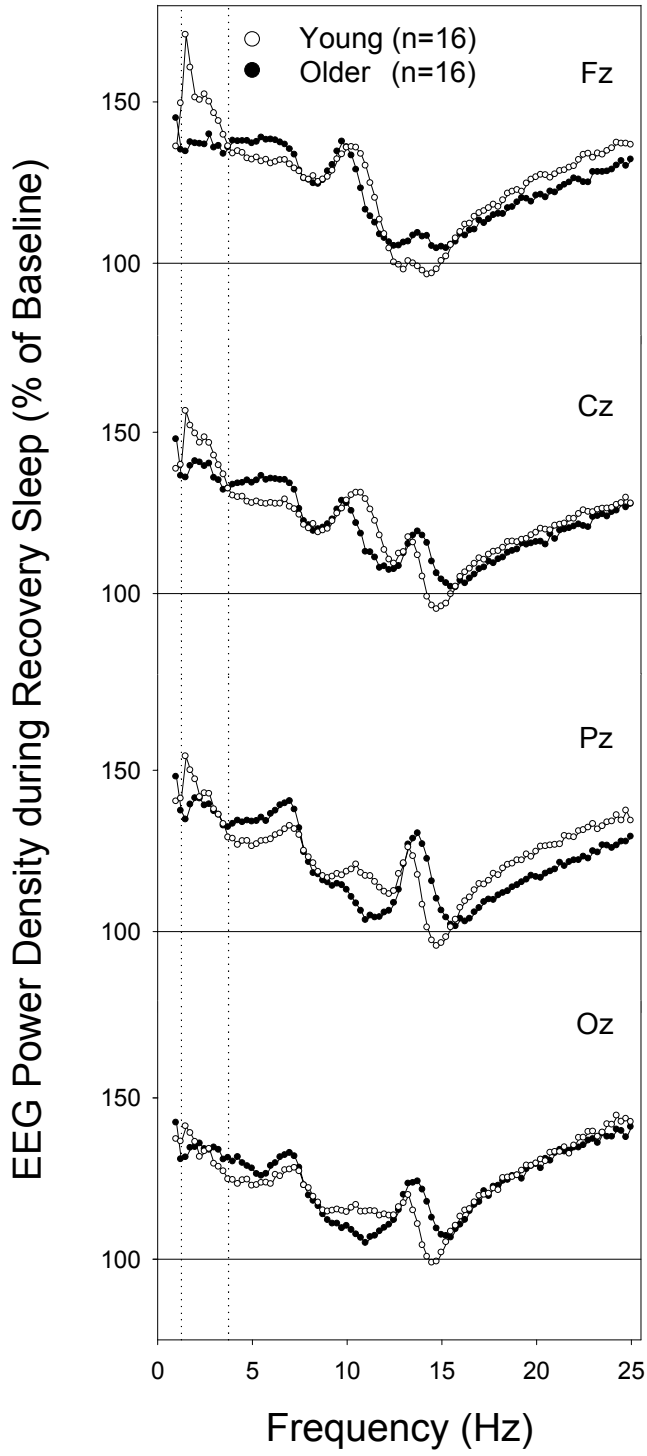

Figure 2

Relative sleep EEG spectra during NREM sleep expressed as percentage of the baseline night (=100%) for the young (open circles) and older group (filled circles) along the antero-posterior axis (Fz, Cz, Pz and Oz) and the frequency range of 1–25 Hz. The vertical dotted lines delineate the frequency bins within the delta range (1.25-3.75 Hz), which yielded a significant 2-way interaction in rANOVA with the factors 'age' x 'derivation'.

Age-related differences in regional EEG delta power after sleep loss

A two-way rANOVA 'age' x 'derivation' exhibited significant interaction ($F_{3,90}$=11.3, p<0.0001 on log-ratios) for the relative EEG delta power density to sleep deprivation, indicating a different regional response to elevated homeostatic sleep pressure with age. The main effect of 'age' was not significant (p>0.1), whereas the factor 'derivation' yielded significance ($F_{3,90}$=24.3; p<0.0001). *Post-hoc* comparisons (Mann-Whitney U test) indicated a significant difference in the frontal derivation (p=0.0014) and a tendency (p=0.055) in the central derivation between the older and the young group (Figure 3). Within the age groups there was a significant fronto–occipital gradient in the young between Fz and Cz, Cz and Pz, Pz and Oz (*post-hoc*: Wilcoxon matched-pairs test; p<0.05) and a lack of such a significant gradient in the older group except for Pz and Oz (p<0.05).

Age-related changes in the time course of relative EEG delta power density during the baseline and recovery night

In order to assess age-related modifications in the temporal distribution of EEG delta power along the antero-posterior axis, relative EEG power density in this frequency range was calculated for 2-h intervals throughout the baseline and the recovery night for each age group separately (Figure 4). During both nights, EEG delta power declined over consecutive NREM sleep episodes in young and older subjects. A four-way rANOVA, performed on EEG delta power density (log-ratios; Table 2) yielded a number of significant results, and an almost significant interaction between the factors: 'age' x 'night' x 'derivation' x 'time interval' (p=0.065; uncorrected p-value: p=0.028). A three-way rANOVA with the factors 'age' x 'derivation' x 'night' performed for each time interval separately resulted in a significant interaction during the first and the third quarter of the night ($F_{3,90}$=4.2 and $F_{3,90}$=5.8; p<0.05). *Post-hoc* comparisons revealed that the differences between young and older subjects occurred mainly during the first quarter of the night (Duncan's multiple range test p<0.05 for Fz and Pz). No significant *post-hoc* comparisons resulted during the third time interval (p>0.1 for all derivations). When a separate rANOVA (three-way rANOVA with the factors 'age' x 'night' x 'time interval') for each derivation was

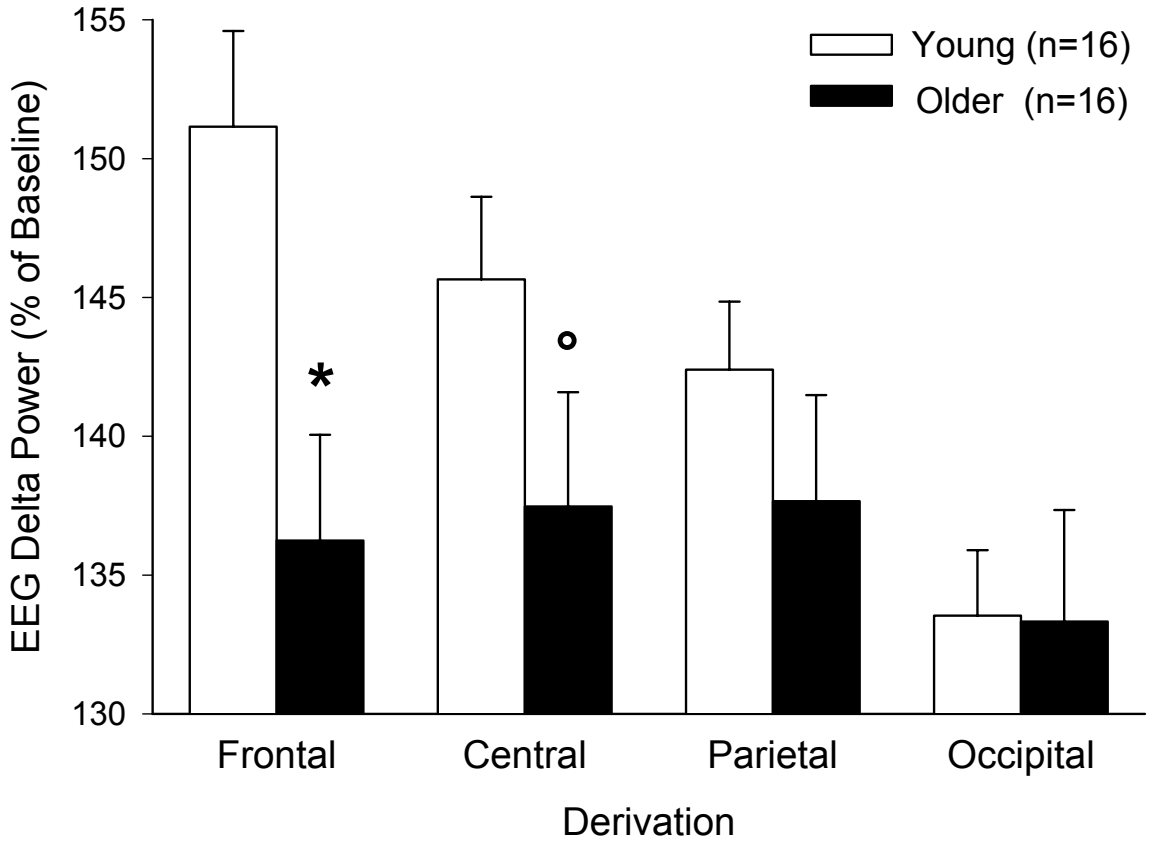

Figure 3

Relative EEG delta power density (1.25-3.75 Hz) during NREM sleep along the antero-posterior axis (Fz, Cz, Pz and Oz) in the young (white bars) and older (black bars) group (mean values + SEM; n=16 for both age groups) after 40-h of sleep deprivation. Values are expressed as percentage of the baseline night. The asterisk indicates a significant difference between young and older subjects and the open circle a tendency (*=p<0.05; °=p<0.1).

performed, a tendency (p<0.1) for the interaction of these factor emerged for the frontal, central as well as the parietal derivation, and a significant interaction for the occipital derivation (p<0.05).

To quantify the 'steepness' of EEG delta activity decrease during the baseline and the recovery night, the difference in relative EEG delta power density between the first and the fourth 2-h interval was calculated for each derivation and age group separately (Figure 5). A three-way rANOVA with the factors: 'age', 'derivation' and 'night' yielded a significant interaction between these factors ($F_{3,90}$=8.0; p<0.05). *Post-hoc* comparisons revealed no significant differences in the steepness of the temporal EEG delta power gradient during the baseline night between both age groups. However, a significantly higher steepness in EEG delta power between intervals 1 and 4 was found during the recovery night in young participants in the frontal derivation (Duncan's multiple range test: p<0.05) and a tendency (p<0.1) for the central and the parietal derivation.

A two-way rANOVA ('age' x 'derivation') calculated for the baseline night separately did not reveal a significant interaction between these factors. However, significant effects were found for the main factors 'age' and 'derivation' ($F_{1,30}$=12.1 and $F_{3,90}$=25.4; p<0.05 for both factors). The same analysis performed for the recovery night yielded a significant interaction for the factors 'age' x 'derivation' ($F_{3,90}$=5.1; p<0.05). This indicates that the age-related dissipation of EEG delta activity during the recovery night depends on brain location (i.e. EEG derivation).

Based on our initial hypothesis, we predicted an age-related reduction in frontal predominance of low EEG components along the antero-posterior axis and a shallower decline of EEG delta power between the beginning and the end of the night. Therefore, we calculated the overall differences in EEG delta activity between time interval 1 and 4 and between the derivation Fz and Oz and between the baseline and the recovery night, resulting in a single measure for the older and young group. This contrast (mean value: 0.61±0.12 SEM for the young and 0.19±0.05 SEM for the older group) was significantly higher in the young (t-test for independent samples; p=0.003).

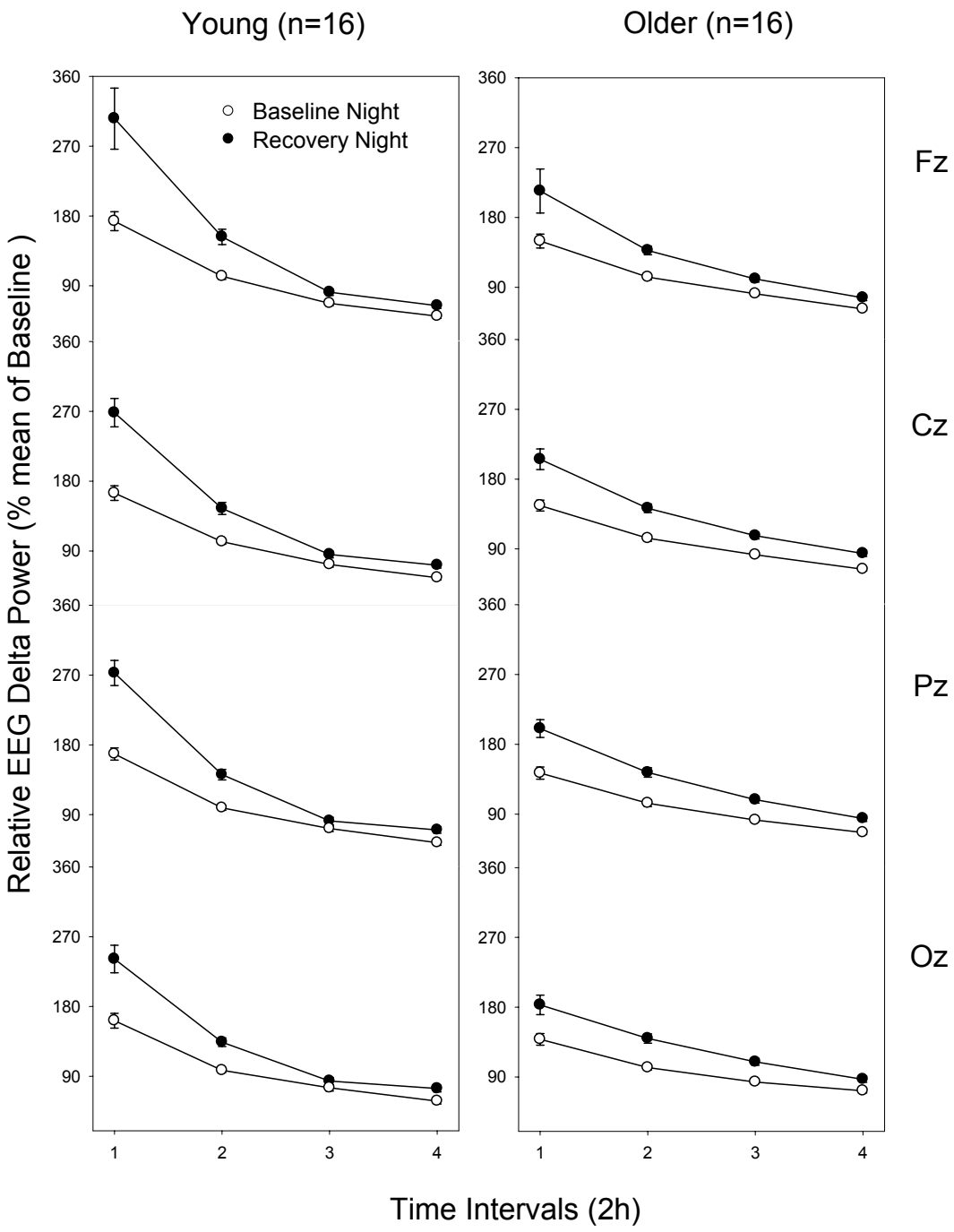

Figure 4

Time course of relative EEG delta power density plotted as percentage of corresponding baseline means during NREM sleep in the baseline (open circles) and recovery night (filled circles) for the young (left hand panel) and older subjects (right hand panels, mean values per 2-h intervals ± 1 SEM, n=16 for both age groups).

Table 2

A four-way rANOVA performed on relative EEG delta activity values during the baseline and recovery night for the young and older group with the factors: ‘age’, ‘night’, ‘derivation’, ‘time interval’

Effect	F-values	df	P-values (H-F)
Night	287.3	1,30	<0.001
Night x Age	0.7	1,30	=0.406
Derivation	3.3	3,90	=0.058
Derivation x Age	3.6	3,90	<0.05
Time Interval	216.9	3,90	<0.001
Time Interval x Age	10.2	3,90	<0.001
Night x Derivation	8.3	3.90	<0.001
Night x Derivation x Age	6.7	3,90	<0.05
Night x Time Interval	7.8	3,90	<0.001
Night x Time Interval x Age	2.6	3,90	=0.061
Derivation x Time Interval	28.0	9,270	<0.001
Derivation x Time Interval x Age	2.1	9,270	=0.091
Night x Derivation x Time Interval	3.9	9,270	<0.05
Night x Derivation x Time Interval x Age	2.1	9,270	=0.065

df= degrees of freedom; H-F= Huynh-Feld corrected

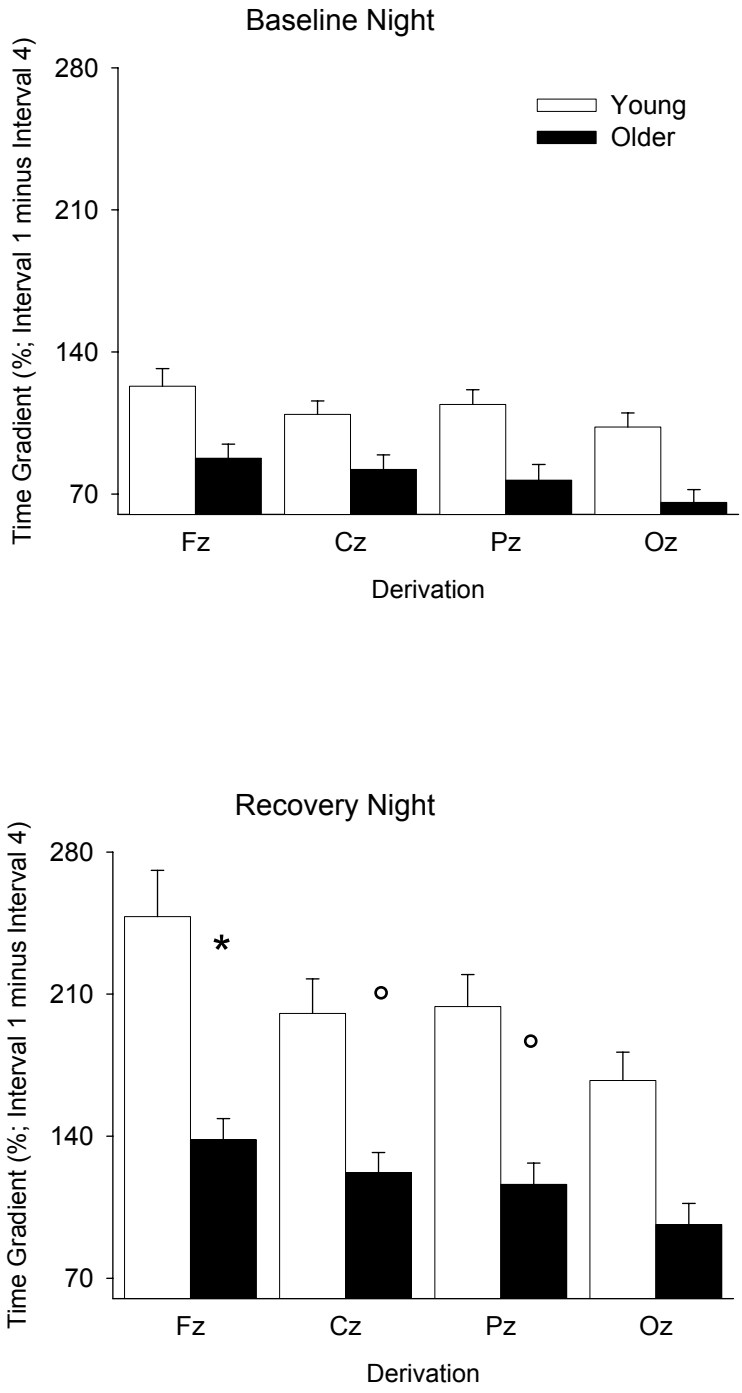

Figure 5

The difference in EEG delta activity (1.25-3.75 Hz) between the first and the fourth 2-h interval of the corresponding night (i.e. each value is expressed as mean of the corresponding baseline) along the antero-posterior axis (Fz, Cz, Pz and Oz). The upper panel shows the values for the baseline and the lower panel those for the recovery night. Black bars=older, white bars=young subjects (+ 1 SEM; n=16 in both age groups). Asterisks indicate significant *post-hoc* comparisons between the young and older subjects and the open circles tendencies (* =p<0.05; ° =p<0.1).

Discussion

We could confirm the hypothesis that the response to elevated sleep pressure during a subsequent sleep episode is attenuated in frontal brain areas with age. The relative increase in EEG delta power density after sleep deprivation declined significantly along the antero-posterior axis in the young, and this decline was no longer present in the older group. Furthermore, dissipation of homeostatic sleep pressure during the recovery night, as indexed by EEG delta activity, revealed a significantly less profound decrease in the older age group when compared with the young.

Age-related homeostatic response to sleep loss

Our data confirmed previous findings of a relative increase of EEG power in the delta, theta, low alpha and the lower spindle range as well as a decrease in the higher spindle range in response to extended wakefulness in the young age group (Borbély *et al.*, 1981; Dijk *et al.*, 1987a; Feinberg *et al.*, 1987; Dijk *et al.*, 1993; Finelli *et al.*, 2000; Knoblauch *et al.*, 2002). An increase of SWS during the recovery night after sleep deprivation in healthy middle aged and older subjects had been reported in earlier studies (Webb, 1981; Carskadon and Dement, 1985; Reynolds *et al.*, 1986; Brendel *et al.*, 1990) but the absence of sleep EEG spectral analysis and the heterogeneity of these study designs does not allow for a direct comparison with our data. More recent studies in older and middle-aged volunteers also reported results derived from EEG spectral analysis (Dijk *et al.*, 1999b; Dijk *et al.*, 2000) as well as after 25 hours of sleep deprivation (Gaudreau *et al.*, 2001b; Drapeau and Carrier, 2004). Our results corroborate those findings, in that older participants exhibited significant lower absolute SWA levels during both the baseline and the recovery night when compared to the young. Furthermore, both age groups in our study responded with a significant increase in SWA during the recovery night after sleep deprivation which has also been reported previously for an older (Dijk *et al.*, 1999b; Dijk *et al.*, 2000) and a middle-aged group of study participants (Gaudreau *et al.*, 2001b; Drapeau and Carrier, 2004). However, none of the previously cited studies reported age-related changes after sleep deprivation over a broad frequency range (0.75-25 Hz). We have evidence that both the young and older age group responded to sleep deprivation with a significant increase in EEG power density in a broad frequency

range during the recovery night - in fact, with the exception of the high spindle range, all frequency bins between 0.75-25 Hz were affected. Targeting the focus more on the markers of sleep homeostasis, the rebound of EEG activity during recovery sleep was further analysed in the EEG delta range. The relative increase of EEG power density in this frequency range derived from a central derivation (Cz) did not significantly differ between age groups when averaged over the entire night. This is in good accordance with a previous study, where the EEG was recorded from central derivations (C3 and C4; Dijk *et al.*, 1999b; Dijk *et al.*, 2000). This supports the interpretation that the responsiveness of the sleep homeostat is still operational in healthy ageing, but at lower absolute EEG delta activity levels. In contrast, another study has reported a less intense homeostatic response of SWA after a 25-h sleep deprivation in middle-aged healthy subjects (Gaudreau *et al.*, 2001b; Drapeau and Carrier, 2004). This divergence to our and other findings may be due to the fact that first, the sleep deprivation episode was shorter and second, recovery sleep started at a different circadian phase (i.e. in the morning). Taken together, at first glance, the relative homeostatic response in SWA to high sleep pressure seems not to be altered by age. However it remains to be elucidated if the build-up of homeostatic pressure during the preceding episode of wakefulness is likewise similar in these age groups.

Age-related regional differences of EEG delta activity

The assessment of the regional distribution of EEG power density in the delta range of young participants exhibits an antero-posterior gradient with highest values in frontal brain regions during baseline sleep episodes (Werth *et al.*, 1997; Knoblauch *et al.*, 2002). An age-related attenuation of this gradient has been reported, with a parietal EEG delta dominance in aged healthy volunteers during baseline sleep episodes (Landolt and Borbély, 2001). To our knowledge, the present study is the first looking at age-related changes in the antero-posterior EEG delta gradient after sleep loss. We found a clear frontal diminution of relative EEG delta power density in the older participants, which was in sharp contrast to the young cohort, who responded to sleep loss with enhanced frontal activity in this frequency range. The function of enhanced EEG delta activity after an extended duration of prior wakefulness is not fully understood, but several explanations have been pursued. It

has been suggested that enhanced EEG delta activity after sleep loss represents a recuperative effect of slow wave sleep in cerebral brain areas intensively stimulated during daytime (i.e. 'frontal tiredness', Horne, 1993; Harrison and Horne, 1998). In terms of mechanisms, enhanced EEG delta activity after sleep deprivation could reflect more hyperpolarised thalamocortical and cortical neurons, which serve to protect the brain from incoming sensory stimuli and therefore allow more 'deep' sleep (Steriade *et al.*, 1993; Mc Cormick and Bal, 1997). Along these lines and based on animal data (Cirelli *et al.*, 1996; Cirelli and Tononi, 2000), Tononi and Cirelli hypothesised that enhanced synaptic potentiation (i.e. structural changes in synaptic strength) in cortical circuits occurring during wakefulness is positively correlated with the enhancement of SWA during the following sleep episode (Tononi and Cirelli, 2003). Our data may indirectly suggest that the synaptic potentiation rate in frontal brain areas during wakefulness decreases with age, resulting in a concomitant diminution of SWA increase in the respective brain areas during sleep.

In brain imaging studies, a negative co-variation of rCBF and EEG delta activity has been observed in frontal regions of the cortex (Hofle *et al.*, 1997; Maquet *et al.*, 1997). Decrease of rCBF in frontal brain areas, particularly the anterior cingulate cortex (Paus *et al.*, 1997; Paus, 2000), may be associated with attenuation of the cortical arousal system during sleep (Hofle *et al.*, 1997). The diminished frontal predominance of EEG delta activity in the older group after extended wakefulness can thus be interpreted as a selective age-related 'alleviated dampening' of cortical arousal during sleep. Such a lower arousal threshold during sleep could underlie the typical age-related increase in sleep fragmentation (Miles and Dement, 1980; Bliwise, 1993; Dijk *et al.*, 1999a; Dijk *et al.*, 2001). Whether the regional amount of SWA during sleep is determined in each brain region not only by the duration but also by the intensity of activity during prior wakefulness, has not yet been unambiguously clarified. Recently, it has been reported that the total amount of SWA was not affected by the level of mental workload preceding the sleep episode in humans, however, EEG recordings were only taken from a single central derivation (De Bruin *et al.*, 2002). Another study in humans demonstrated a relatively small local augmentation of EEG delta power density (0.75-4.5 Hz) during subsequent NREM sleep after unilateral activation of the left somatosensory cortex during wakefulness (Kattler *et al.*, 1994). Investigation of continuous auditory stimulation during

wakefulness yielded both an increase in power density in the alpha and spindle frequency range, and changes in fronto-temporal coherence over a broad frequency range during subsequent SWS (Cantero *et al.*, 2002). In animal studies, experience-dependent slow wave sleep generation has been found in light-deprived cats (Miyamoto *et al.*, 2003). In rats whose whiskers were cut on one side in order to reduce the sensory inputs in the contralateral cortex a shift of the interhemispheric asymmetry of EEG power (0.75-6 Hz) during NREM sleep was reported (Vyazovskiy *et al.*, 2000). Hence, there is mounting evidence of a 'use' or 'experience'- dependent process which occurs during sleep and affects EEG synchronisation. This has led to the hypothesis that the sleep EEG shows use-dependent characteristics and reveals presumably not global but local processes (Krueger and Obal, 1993; Borbély, 2001). A very recent study has brilliantly demonstrated selectively localised SWA induction triggered by a learning task (Huber *et al.*, 2004) providing further evidence for a link between sleep and learning. The significance of the here reported altered pattern of frontal EEG delta activity during recovery sleep in the elderly in association with the use-dependent activity of frontal areas needs further clarification. As all of the aforementioned studies on use- or experience dependent aspects on sleep regulation reported small and short-lasting effects (i.e. at the beginning of the sleep episode), we were interested in the temporal characteristics of the observed age-related disappearance of frontal predominance after sleep loss.

EEG delta activity dissipation during the baseline and recovery night

Our data confirm and extend a previous result in older volunteers showing that the decline in relative EEG delta activity after sleep deprivation is shallower than in young study participants (Dijk *et al.*, 1999b; Dijk *et al.*, 2000). The reduced decay rate of EEG delta activity has been postulated to reflect an age-related alteration of the dynamics of the homeostatic regulatory process (Dijk *et al.*, 1999b). Another study in older volunteers (age range: 57-64 years) reported that the decline of relative SWA over the course of the night flattened even during baseline (Landolt *et al.*, 1996; Landolt and Borbély, 2001), as we also found. However, in the young volunteers, this time gradient of SWA dissipation during the recovery night increased significantly

more steeply in frontal areas compared to the older participants, whereas no significant changes were present in more parietal regions. Taken together, the regional dissimilarity between young and older subjects in the rebound of EEG delta power was most pronounced in the first part of the recovery night. However, the reduced steepness of the time course of EEG delta activity in the older group represents a more long-lasting effect, already measurable during the baseline night. This further supports the interpretation of intact sleep homeostatic regulation with age albeit on a lower EEG amplitude level.

Conclusion

The activation of sleep regulatory processes by an extension of wakefulness from 16 to 40 hours revealed a smaller increase of relative EEG delta power density in frontal brain areas in older study participants, particularly during the first part of the recovery night. This is in accordance with the assumption that frontal brain areas are subjected to a double vulnerability – the ageing process *per se* and the short-term response to sleep loss.

Acknowledgements

We are very grateful to our technicians Claudia Renz, Marie-France Dattler, Giovanni Balestrieri and the student shift workers for their help in data acquisition. We thank all the study participants for their compliance in a demanding study. This research was supported by Swiss National Foundation Grants START #3100-055385.98 and 3130-054991.98 to CC and the Velux Foundation (Switzerland).

CHAPTER 3

AGE-RELATED ATTENUATION OF THE EVENING CIRCADIAN AROUSAL SIGNAL IN HUMANS

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Published in: Neurobiology of Aging (2005), 26: 1307-1319

Summary

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

Introduction

The mammalian circadian system, one of whose major functions is the regulation of sleep and wakefulness, is governed by the hypothalamic suprachiasmatic nuclei (SCN; Moore and Eichler, 1972; for reviews see Weaver, 1998; Van Esseveldt *et al.*, 2000). The differential neuronal activation of the SCN is dependent on various input signals from the environment such as light and social cues (Duffy *et al.*, 1996; Klerman *et al.*, 1998). Lesions of the SCN eliminate circadian sleep-wake cycles in animals as well as circadian regulated endocrine functions, motor activity and core body temperature (CBT) rhythms (Edgar *et al.*, 1993). The rare human lesion data support these findings (Schwartz *et al.*, 1986; Cohen and Albers, 1991; Bloch *et al.*, 2005). How the SCN communicates within its sub-sections and with the rest of the brain and body to elicit physiological and behavioural function adequately synchronized to the appropriate time of day is still the subject of intense ongoing research (Deboer *et al.*, 2003; Schwartz and Meijer, 2004; Deurveilher and Semba, 2005).

The two process model of sleep and wakefulness predicts the day-to-day synchronization of an organism to its environment by the interaction of a circadian (C) and a homeostatic process (S; Borbély, 1982; Daan *et al.*, 1984). Two hallmarks of this system are remarkable: based on experimental findings and theoretical assumptions (Lavie, 1986; Strogatz *et al.*, 1987; Dijk and Czeisler, 1994) a circadian arousal signal in the evening opposes the homeostatic drive for sleep shortly before melatonin onset and disappears prior to sleep onset (the ‘wake maintenance zone’). The opposite occurs in the early morning shortly after the CBT nadir, when the homeostatic need for sleep is lowest and yet the circadian drive for sleep appears high. Therefore, the progressive increase in the circadian drive for sleep in the course of the nocturnal sleep episode counteracts the dissipation of homeostatic sleep pressure associated with consolidated sleep (Lavie, 1986; Strogatz *et al.*, 1987; Dijk and Czeisler, 1994).

Evidence for this model comes from so-called forced desynchrony (FD) studies (Czeisler *et al.*, 1990) where the volunteers’ sleep-wake cycles are scheduled to artificial days longer or shorter than 24 hours (e.g. 28 or 20 hours). Under such conditions, the circadian pacemaker can no longer entrain to the imposed sleep-

wake cycle and ‘free-runs’ with its own endogenous period. This means that sleep occurs at all different circadian phases, permitting assessment of the contribution of the circadian pacemaker and the sleep-wake dependent process to sleep propensity (Czeisler and Khalsa, 2000).

With age, some aspects of the sleep-wake cycle undergo well-known changes, such as shallower nocturnal sleep with more arousals, less slow wave sleep (SWS) and the prevalence of daytime naps (Buysse *et al.*, 1992; Bliwise, 1993). Most (Weitzman *et al.*, 1982; Van Coevorden *et al.*, 1991; Czeisler *et al.*, 1992), but not all studies (Monk *et al.*, 1995; Zeitzer *et al.*, 1999) report a decline in the amplitude of circadian markers e.g. melatonin, CBT and cortisol. The elderly usually (Duffy *et al.*, 1998; Duffy *et al.*, 2002), but not always (Monk *et al.*, 1995) have earlier habitual bed- and wake-up times with an advanced circadian phase in relation to their CBT minimum (and melatonin secretion maximum), when compared to young volunteers. The endogenous circadian period remains rather stable with age (Czeisler *et al.*, 1999). It is not yet clear whether the circadian or homeostatic aspects of sleep regulation are more affected by age. We have recently obtained evidence that although the sleep homeostat retains its capacity to respond to sleep loss in Ageing, there is a significant age-related decrease of frontal delta predominance in this response (Münch *et al.*, 2004). Even though circadian facets of sleep regulation seem to be affected by age (Dijk *et al.*, 1999a), we do not know whether ageing *per se* causes changes in the circadian regulation of sleep and wakefulness, or whether they are rather a consequence of a modified regulation of circadian signalling downstream (or both). Moreover, to what extent the presumed dampened amplitude of circadian rhythms (such as melatonin, cortisol, CBT) is associated with changes in other physiological and neurobehavioral functions, has not been unambiguously clarified.

We therefore hypothesized both an age-related decrease in the circadian secretion of melatonin and a less pronounced day-night difference between sleep and wakefulness in the older group. This implies more sleep and higher subjective sleepiness levels during the day and less sleep and lower subjective sleepiness levels during the night. We tested our hypotheses in a 40-h multiple nap protocol carried out under stringent constant posture conditions, with sleep and wakefulness occurring at different circadian times, using quantitative sleep EEG analysis along the

antero-posterior axis, continuous assessment of subjective sleepiness and measurement of melatonin secretion.

Methods

Screening procedure

All study participants were recruited via advertisements at different Swiss universities and in newspapers. Only candidates with a Pittsburgh Sleep Quality Index (PSQI) score ≤5 (Buysse *et al.*, 1989) and no extreme chronotype, i.e. ratings between 14 and 21 points on the morning-evening-type (M/E) questionnaire were selected (Torsvall and Åkerstedt, 1980). Additionally, all potential study participants were asked about their sleep quality, life habits and health state. Exclusion criteria were: smoking, medication or drug consumption, shift work within the last 3 months and transmeridian flights within one month prior to the study. Each study volunteer underwent a physical examination, an interview, a neuropsychological test battery (only for the older group) and a polysomnographically recorded adaptation night. Only volunteers with a clinical sleep EEG scoring without any pathological findings [apnoea/hypopnoea-index (AHI) <10; periodic leg movements (PLM) <10/h] were included in the study. All participants gave written informed consent. The study protocol, the screening questionnaires and consent form were approved by the local Ethical Committee and conformed with the Declaration of Helsinki.

Study participants

Seventeen healthy young (nine female, eight male, age range 20-31 years, mean: 25.0±3.3 SD) and fifteen healthy older volunteers (seven females, eight males, age range 57-74 years, mean: 65.1±5.6 SD) were selected for the study. The mean PSQI value was 2.1±1.3 SD for the young and 3.4±1.7 SD (t-test: $p<0.05$) for the older volunteers. The ratings on the M/E questionnaire were slightly but significantly higher (earlier chronotype) in the older (mean 18.8±3.0 SD) than in the young group (mean 16.4±3.2 SD; t-test: $p<0.05$). The mean body mass index (BMI) was 21.5±1.6

(mean±SD) for the young and 23.3±2.1 for the older volunteers (t-test: $p<0.05$). All subjects were free from medical, neurological, psychiatric and sleep disorders and were non-smokers without any drug abuse. The latter was verified for the young group by an urinary toxicological analysis, sensitive for amphetamines, benzodiazepines, opiates and tetrahydrocannabinol (Drug-Screen Card Multi-6®, von Minden GmbH, Moers, Germany). Except for five young female subjects taking oral contraceptives, none took any medication. Young females started the study on day 1-5 after menses onset during the follicular phase of their menstrual cycle.

Study design

One week prior to study begin (baseline week) participants were instructed to keep their individual bed- and wake times within a range of ± 30 minutes (compliance controlled by a wrist activity monitor, Cambridge Neurotechnologies®, Cambridge, UK and sleep logs). They were also required to abstain from excessive caffeine and alcohol consumption as well as heavy physical exercise. After the baseline week, the participants reported to the laboratory in the evening before day 1 (Figure 1). The timing of their sleep–wake schedule was calculated such that the 8-h sleep episode was centred at the midpoint of each subject's habitual sleep episode as assessed by actigraphy and sleep logs during the baseline week. Habitual bedtimes did not vary significantly between groups (young: 23:34±56 min vs. older: 23:11±40 min; mean± SD; $p=0.2$, t-test; mean difference: 23 min). The study started with an 8-h adaptation night in the laboratory. The following 16 hours of wakefulness on day 1 were used to adjust the subjects to the experimental dim light condition (<8 lux). During the morning a blood sample was taken from the older participants in order to verify both a normal haemogram and physiological coagulation. The older volunteers received a heparin low-dose injection on the three consecutive days of each study block (Fragmin® 0.2ml, 2500 IE/Ul, Pharmacia AG, Dübendorf, Switzerland) in order to prevent any venous thrombosis. In the afternoon of day 1, all subjects were prepared for continuous polysomnographic recording. After a second 8-h sleep episode (baseline night), the subjects followed scheduled 75/150-min sleep-wake cycles during a 40-h protocol under constant conditions. This included constant recumbent

body posture, no time cues, dim light conditions (<8 lux) during wakefulness and no light (0 lux) during sleep, and regular small isocaloric meals and water (for details of the method see Czeisler *et al.*, 1985; Cajochen *et al.*, 1999b).

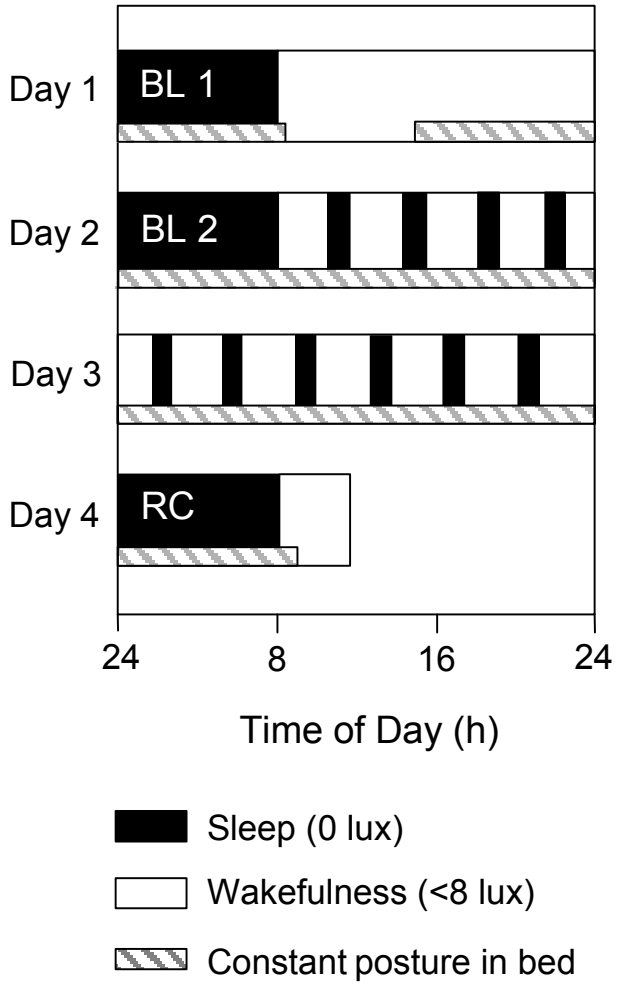

Figure 1

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

EEG sleep recordings and spectral analysis

Sleep was polysomnographically recorded with the VITAPORT ambulatory system (Vitaport-3 digital recorder, TEMEC Instruments B.V., Kerkrade, the Netherlands). Twelve EEGs, two electrooculograms, one submental electromyogram and one electrocardiogram were recorded. All signals were low-pass filtered at 30 Hz (4^{th} order Bessel type anti-aliasing, total 24 dB/Oct) at a time constant of 1 s. After online digitization by using a 12 bit AD converter (0.15 μV/bit) in the range of 610 μV and a sampling rate at 128 Hz for the EEG, the raw signals were stored on a Flash RAM Card (Viking, USA) and later downloaded to a PC hard drive. Sleep stages were visually scored per 20-s epochs (Vitaport Paperless Sleep Scoring Software) according to standard criteria (Rechtschaffen and Kales, 1968).

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

Sleep stages

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

age-group was analyzed with the Friedman-test, and group differences were calculated with the mean values over 11 naps (Mann-Whitney U test).

Subjective sleepiness ratings

Subjective sleepiness was assessed on the Karolinska Sleepiness Scale (KSS) from 1 (very alert) to 9 (very sleepy; Gillberg *et al.*, 1994) every ~30 min during scheduled wakefulness. Missing data were linearly interpolated. KSS values were collapsed into 1.25 hourly bins per subject before averaging over subjects. The very first sleepiness rating taken immediately after the naps was not included in this average in order to exclude sleep inertia effects (Kräuchi *et al.*, 2004).

Salivary melatonin

Saliva collections were scheduled during wakefulness at the same time intervals (every ~30 min) as the subjective sleepiness ratings (KSS). A direct double-antibody radioimmunoassay was used for the melatonin assay (validated by gas-chromatography-mass spectroscopy with an analytical least detectable dose of 0.65 pm/ml; Bühlmann Laboratories, Schönenbuch, Switzerland; Weber *et al.*, 1997). As for the sleepiness ratings, missing data were linearly interpolated and all melatonin values were collapsed into 1.25 hourly bins per subject before averaging over subjects. For calculating mean melatonin levels during the active secretion time, values of all samples between the upward- and downward-mean crossing points were averaged per subject and age group (see Table 1).

Classification of day and night naps

The first 75 min after lights off in the recovery night were considered as an additional nap. In order to compare EEG sleep spectra between ‘night’ and ‘day’ naps, the 24-h mean melatonin concentration (between hours 5 and 29 of the 40-h protocol) was calculated for each subject. This overall mean was 9.1±5.4 pg/ml for the young and

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

Statistics

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

Table 1

Melatonin analyses for young (n=17) and older (n=15) volunteers separately (mean±SD).

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

Results

Sleep stages during the naps

Sleep measures derived from visual scoring are summarized in Table 2. There were no significant age differences in TST, SE, WALO and stage 1 (Mann-Whitney U test: $p>0.1$). Older volunteers had significantly less SWS (stage 3+4) and REM sleep during the nap protocol than the young at the cost of significantly more stage 2, also reflected in more NREM sleep (p for all ≤ 0.006). SL1 revealed no significant age difference ($p=0.98$), whereas RL was significantly shorter ($p<0.001$) and SL 2 tended to be longer in young volunteers ($p=0.07$). *Post-hoc* comparisons (Mann-Whitney U test) revealed that the young participants slept significantly less during nap 4 and 10 and significantly more during nap 5 and 8 ($p<0.036$; Figure 2A). The same results were obtained for SE ($p<0.036$; Figure 2E) with a tendency in the latter during nap 7 ($p<0.073$). This was also reflected in the duration of wakefulness (WALO) where older subjects had significantly less wakefulness during the wake maintenance zone (nap 4 and 10) and more during naps 5, 7 and 8 than the young ($p<0.032$; graph not shown). Older subjects had more NREM sleep during naps 1, 4, 7 and 10 ($p<0.036$; Figure 2B). In parallel, the elderly had significantly more stage 2 (Figure 2D) during all naps ($p<0.014$) except for nap 3 and 11. On the other hand, the young participants had more SWS (Figure 2C) during naps 3 ($p<0.036$), 5, 8 and 9 (with a tendency during naps 2, 7 and 11; $p<0.073$) than the older subjects. SL2 (Figure 2F) was shorter for the older group during nap 1 and during the wake maintenance zone (nap 4, 10) as well as during nap 7 ($p<0.036$). The significant longer SL2 in nap 7 for the young group could be explained with the very short RL in this nap. RL was longer for the older group during nap 1, 7, 8 ($p<0.04$; with a tendency during nap 3 and 6 $p<0.082$; Figure 2G), which was exactly the time of day when most REM sleep (Figure 2H) occurred. The older subjects had significantly less REM sleep during naps 2, 7, 8 ($p<0.04$) and a tendency during the first nap ($p<0.082$) although the circadian modulation of REM sleep was clearly present in both groups.

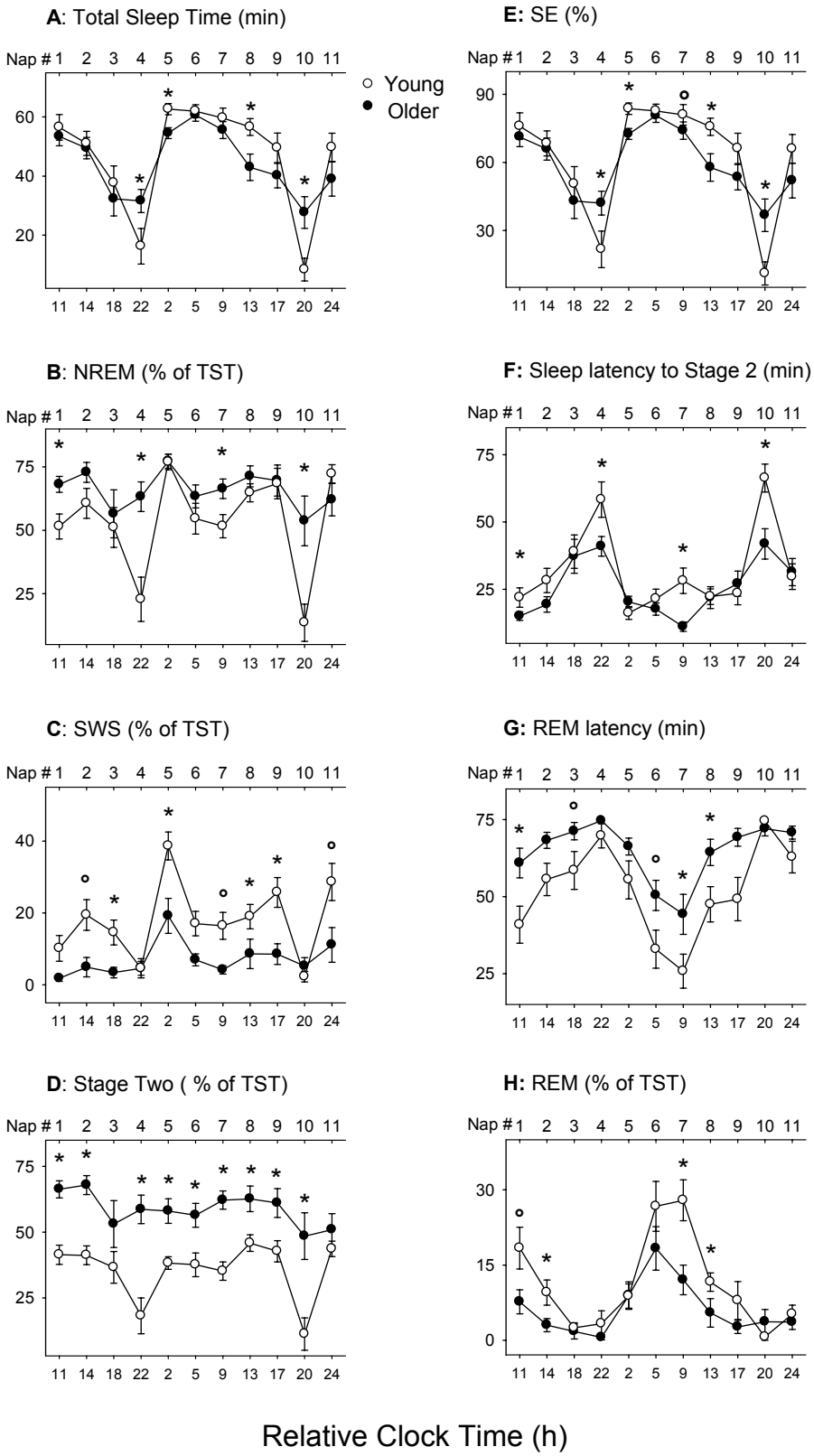

Figure 2

Time course of sleep stages (2A-2H) across the 40-h nap protocol. Open circles: young volunteers (n=17), filled circles: older volunteers (n=15; mean ± SEM), *=p<0.05; °=p<0.1.

Table 2

Sleep stages derived from visual scoring for both age groups, averaged across nap 1-11. Values are indicated ± SEM, n=17 for young and n=15 for older subjects.

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

TST= total sleep time (min; stages 1-4 + REM sleep); SE= sleep efficiency [%; (TST/time after lights off) x 100]; WALO= wake after lights off [%; (wakefulness + movement time)/time after lights off]; SWS= slow-wave sleep (% of TST; stage 3 + 4); NREM sleep= Non-rapid eye movement sleep (% of TST; stage 2 - 4); SL1 (min)= sleep latency to stage 1; SL2 (min)= sleep latency to stage 2; RL (min)= latency to REM sleep (after sleep onset); *p*-values between age groups (fourth column; Mann-Whitney U test) as well as in Chi-square (χ^2) and *p*-values from the Friedman Test for each group (dF=10) are indicated (fifth and sixth column). *=p<0.05 and **=p<0.001; °=p<0.1; ns=not significant.

Time course of TST for young and older volunteers within naps

The time course of sleep within the naps and TST of each nap sequence is shown as a function of relative clock time in Figure 3. There are sharp blue 'valleys' in the left panel which illustrate no or very little sleep for the young group at these specific time points in the evening (during naps 4 and 10). During the other naps, TST was relatively high in the young as indicated by the more long-wavelength colors (yellow and orange). The right hand panel of Figure 3 illustrates the same 3-dimensional interaction of relative clock time, minutes after lights off and the amount of TST per 5-min bin for the older volunteers. There was no significant difference in TST between both age groups, averaged over the entire 40-h nap protocol, but the lack of clear-cut blue valleys during the wake maintenance zone (naps 4 and 10) indicates that the older volunteers were able to sleep significantly more at this time of day even though SL was also longer in these naps (for statistics see Table 2). On the other hand, time intervals with much sleep (4-5 min, yellow and orange) were more scarce in the older than in the younger group, indicating a higher amount of wakefulness during naps outside the wake maintenance zone.

Time course of salivary melatonin, subjective sleepiness and sleep efficiency

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

acrophase of their melatonin secretion. The time course of KSS ratings yielded a significant effect for each age group (Friedman-Test: $p<0.001$; $\chi^2=102.6$ for the older and $\chi^2=171.4$ for the younger group; dF=21). Older volunteers felt significantly sleepier in the late afternoon and evening of the first as well as in the evening of the second day (*post-hoc* comparisons: $p<0.045$, Mann-Whitney U test).

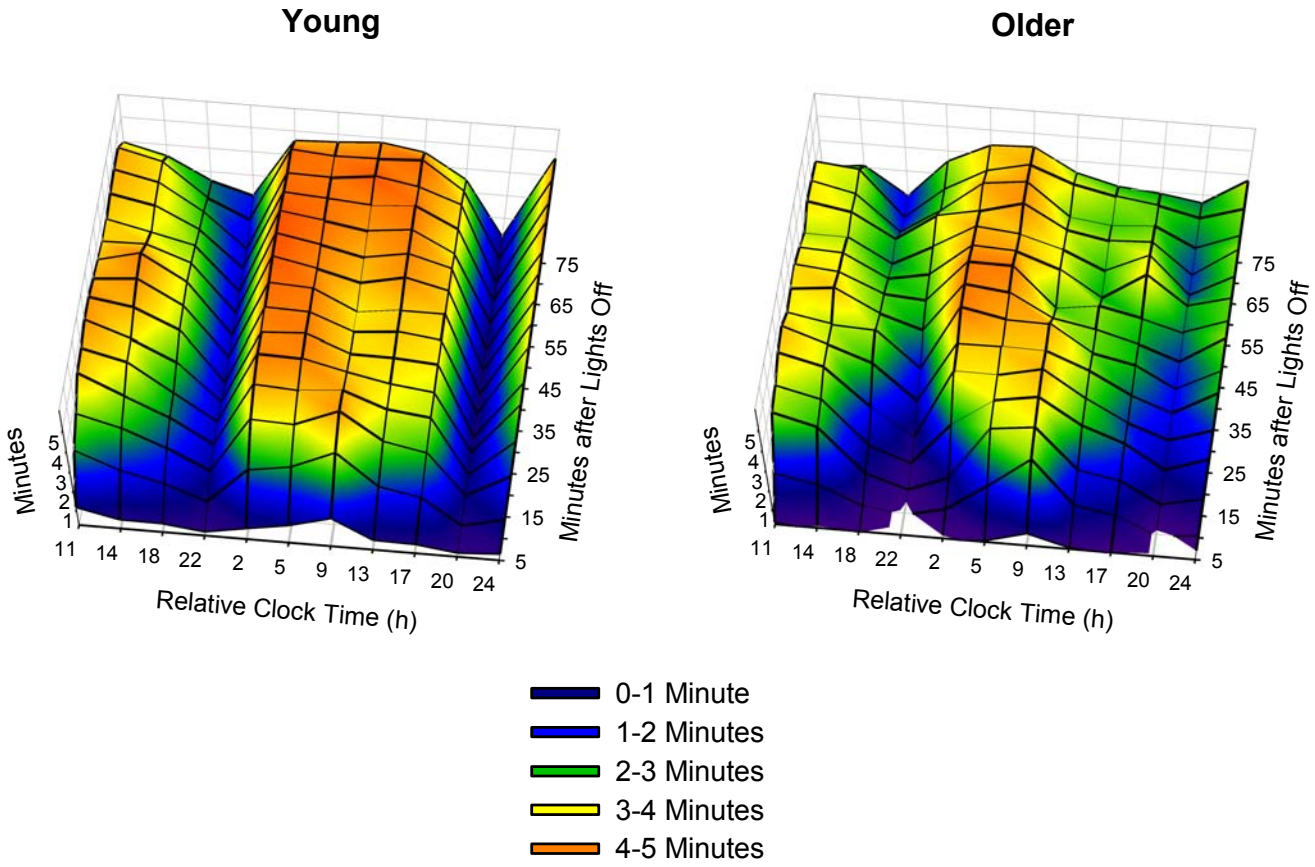

Figure 3

Quasi three-dimensional plots of TST for both age groups. Left panel: young (n=17) and right panel older subjects (n=15). The x-axis represents the averaged mid-nap clock times for both age groups and the y-axis the time course within the respective naps (0-75 min). The z-axis specifies the amount of sleep (TST) per 5-min bin of each nap (min). Short-wavelength colors (blue, green) illustrate less sleep, longer wavelength colors (yellow, orange), more sleep.

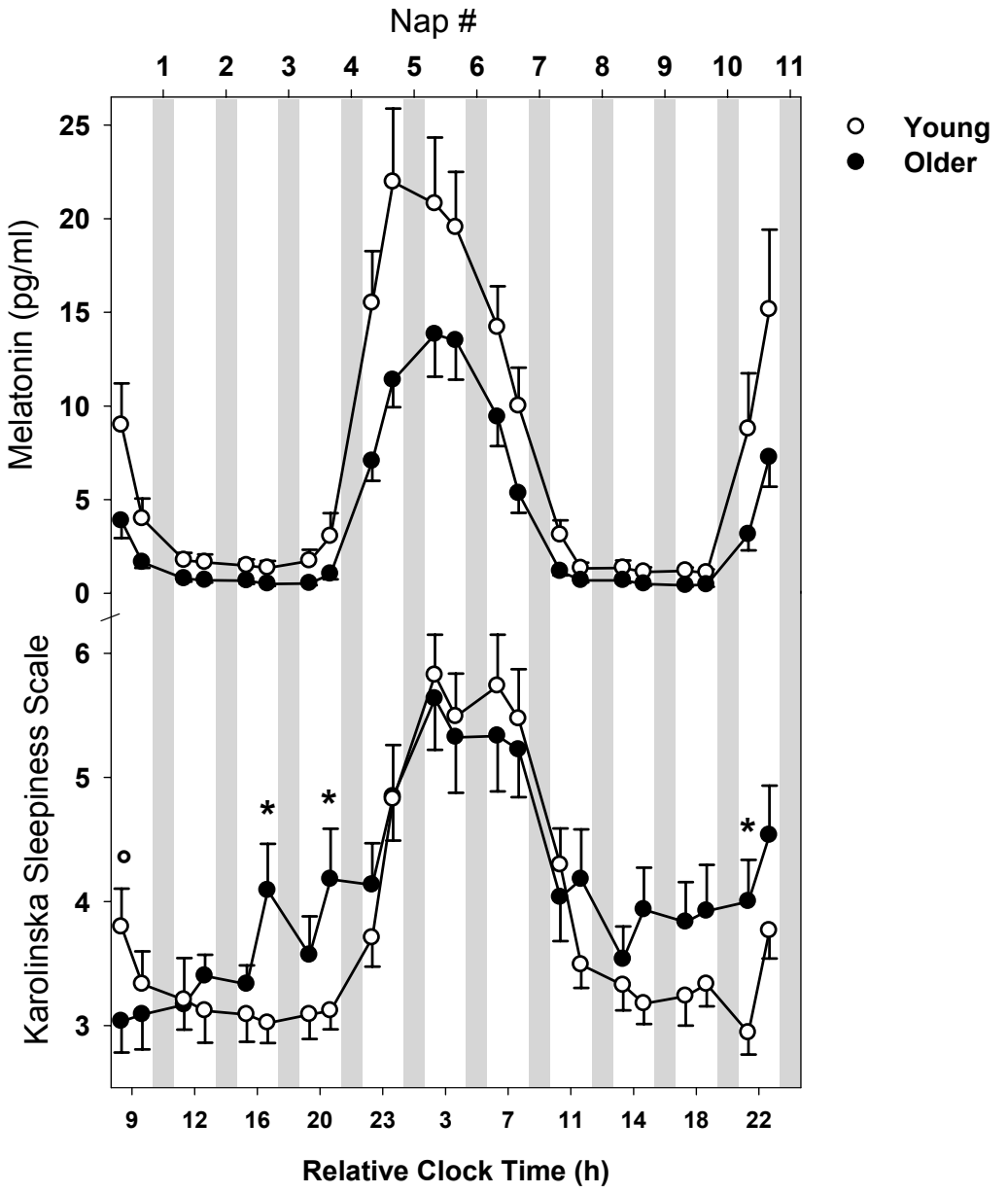

Figure 4

The top panel shows the melatonin secretion during the 40-h nap protocol between young (white circles) and older volunteers (black circles, mean values + or – SEM; n=17 for the young and n=15 for the older). The bottom panel represents subjective sleepiness ratings (KSS) of both age groups during scheduled wake episodes. (*=p<0.05; °=p<0.1).

The young subjects tended to feel sleepier after waking from a night's sleep (first wake episode $p<0.09$). The mean sleepiness ratings averaged across all wake episodes did not significantly differ between groups. (3.8±0.5 SD for the young and 4.1±0.8 SD for the older volunteers; Mann-Whitney U test: $p=0.2$). The time course of subjective sleepiness differed from that of sleep efficiency (or total sleep time Figure 2A). Correlation analyses between these two measures (i.e. the mean of each wake and adjacent sleep episode separately) revealed that the correlation coefficients were relatively low ($-0.2<R<0.3$) and not significant for all the naps (Spearman rank correlation).

Day-night differences in the EEG sleep spectra

The absolute mean of biological day- and night-spectra are illustrated in Figure 5 (left and right hand panel) for both age groups. A significant interaction between the factors 'age', 'derivation' and 'condition' ($p<0.05$, three-way rANOVA; performed on log-transformed data) occurred in the frequency range 0.75-2 Hz, 2.5-2.75 Hz, 3-3.25 Hz, 6.5-7 Hz, 7.25-8.75 Hz, 13.5-14 Hz and 15-15.5 Hz. The day-night differences between both groups were significant in the frequency ranges: 7.75-8 Hz, 8.25-8.75 Hz, 11-14 Hz, 14.5-15.75 Hz. Young volunteers developed an overall higher EEG density power during the biological day and night in the delta (0.5-4.5 Hz), theta (4.5-8.25 Hz), as well as in the spindle range (12-15.25 Hz) in all derivations (main effect of age; $p<0.05$). Figure 6 illustrates the EEG biological night spectra expressed as a percentage of the biological day spectra (=100%). A two-way rANOVA performed on relative EEG values (day/night ratio) of all derivations revealed a significant higher nocturnal EEG activity in the lower alpha (7.75-8 Hz, 8.25-8.75 Hz) and lower spindle range (11-14 Hz) in the younger group, whereas the higher spindle range (14.5-15.75 Hz) yielded significant higher relative values in the older group (main effect of age; $F>4.6$, $p<0.05$). These age differences were significant in all derivations and by visual inspection most pronounced in the parietal derivation. A significant interaction between the factors 'condition' and 'derivation' performed for each age group separately was found in the following frequency ranges for the young volunteers:

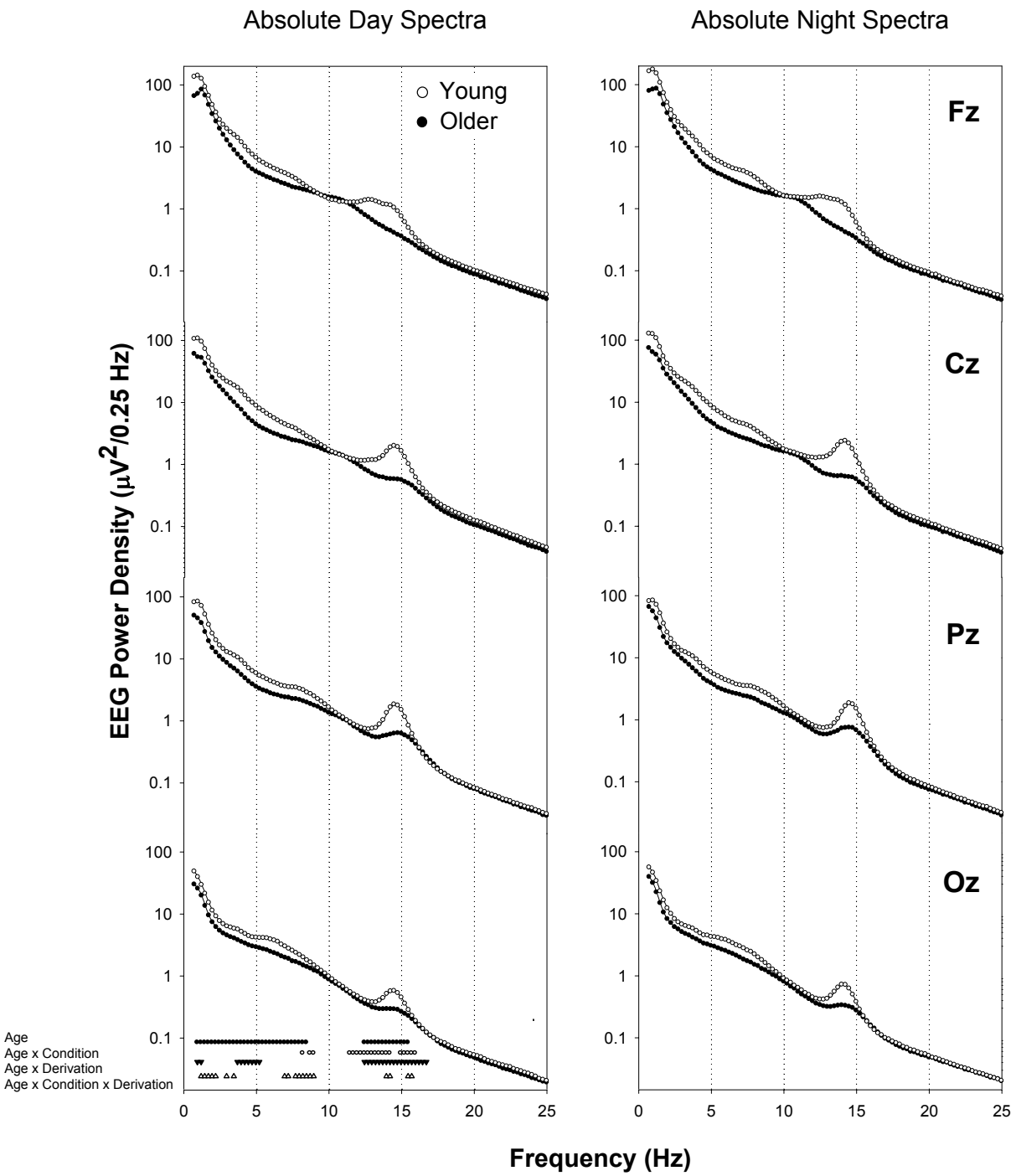

Figure 5

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

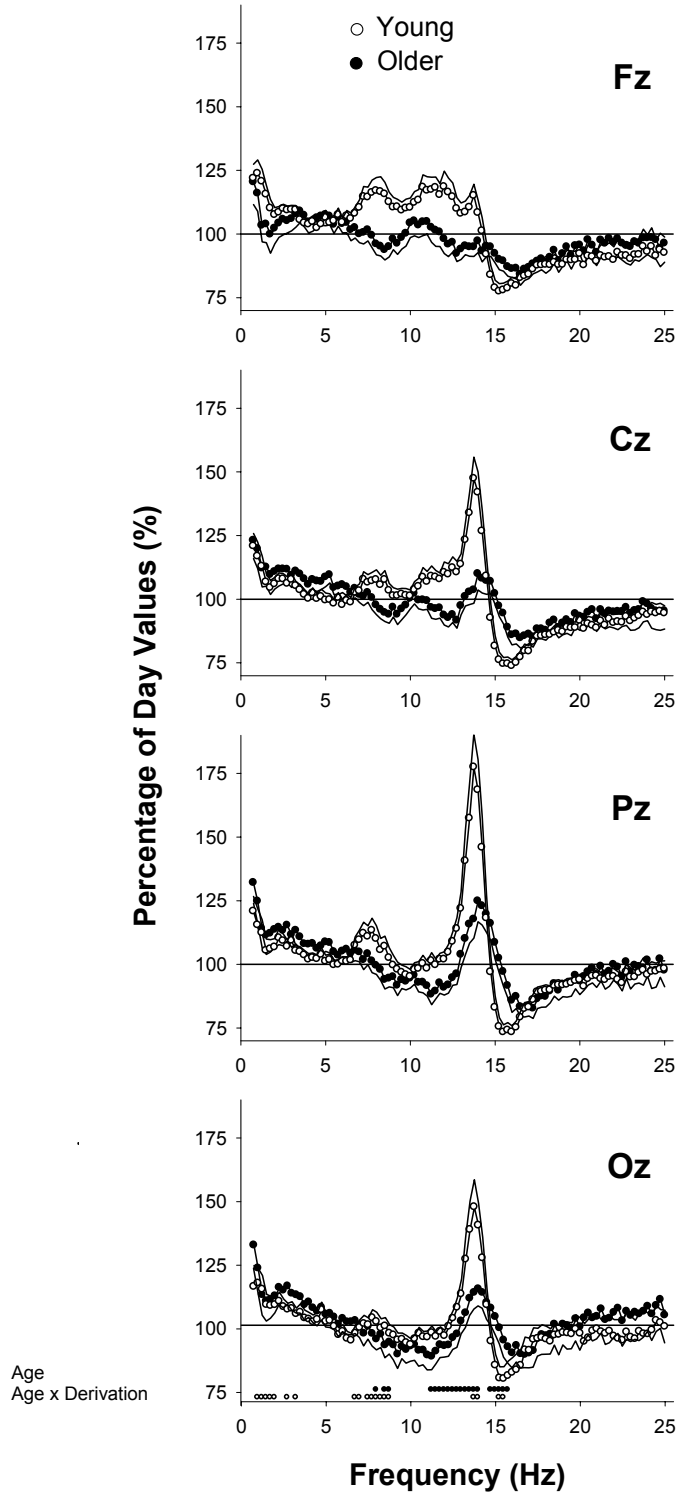

Figure 6

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

5.5-12.5 Hz, 12.75-14.75 Hz, 16.5-17.75 Hz, 18.5-19 Hz and for the older group between: 0.5-0.75 Hz, 1-1.25 Hz, 1.5-2.25 Hz, 2.5-2.75 Hz, 10.75-11 Hz, 12.75-15.25 Hz, 17.25-18 Hz, 18.25-18.5 Hz, 18.75-19 Hz, 19.25-19.75 Hz, 20-21.25 Hz, 21.75-22.25 Hz, 22.5-23.5 Hz, 23.75-24 Hz, 24.25-24.75 Hz (two-way rANOVA, *p* at least <0.05).

Discussion

The study provides quantitative evidence for an age-dependent decrease of the circadian arousal signal in the evening. This is manifested in significantly more sleep in the older group during the wake maintenance zone. Furthermore, older subjects felt significantly more sleepy at circadian times corresponding to the late afternoon and evening (16:00-22:00 h). The study additionally confirmed the hypothesis of an age-related attenuation of melatonin secretion during the biological night in the healthy older group. Relative EEG power density during the biological night (percentage of daytime values) revealed a significant age-related reduction in the lower alpha (7.75-8 Hz, 8.25-8.75 Hz) and in the lower spindle range (11-14 Hz), whereas the relative decrease in EEG power density in the higher spindle frequency range (14.5-15.75 Hz) was significantly less pronounced in the older group during the biological night.

Sleep stages

When the eleven scheduled 75-min naps were averaged, there were no age-related differences in sleep duration (TST) and sleep efficiency. However, the distribution of sleep stages across and within sleep episodes was significantly altered by age, such that SWS and REMS were reduced in favor of stage 2. Our results corroborate findings from a nap-study with ultra short sleep-wake cycles (7/13 min; Haimov and Lavie, 1997), whereby older subjects exhibited a higher sleep propensity (reflected in TST) during the ‘wake maintenance zone’. Our young volunteers slept longer during several naps except for those during the wake maintenance zone, characterized by longer duration of wakefulness (WALO) and longer sleep latencies to stage 2 at this circadian time. On the other hand, neither WALO, nor sleep latency to stage 1 and 2

differed significantly between age groups. This is in contrast to FD studies, where older subjects slept less and were significantly longer awake during scheduled sleep at all circadian phases (Dijk *et al.*, 1999a). One possible explanation might be the age-related vulnerability to the desynchronizing effect of the FD protocol, (i.e. the problems of sleeping at adverse circadian times). This argument is further supported by simulated jet lag and shift work studies where older volunteers show a higher susceptibility to circadian phase misalignment (Moline *et al.*, 1992; Campbell, 1995). Additionally, the higher amount of prior wakefulness (i.e. the wakefulness during scheduled sleep episodes and during the scheduled wakefulness) among the older volunteers in the FD protocol could have led to a modified proportion of sleep/wake cycles and therefore biased the duration and frequency of awakenings in those studies. In this sense, the multiple nap protocol has the advantage of being less masked by such evoked responses, because the frequency of scheduled sleep times was high (every 150 min) and the total duration of sleep episodes of 13.75 hours was long enough to effectively 'counteract' the build-up of homeostatic sleep pressure.

The age-related reduction of SWS in our study was in accordance with many others (Buysse *et al.*, 1992; Landolt *et al.*, 1996) and clearly shows reduced NREM sleep intensity with ageing. Interestingly, the portion of visually scored sleep stage 2 was significantly higher during all but the third and the last nap, which is at variance to other studies (Dijk *et al.*, 1999a) where older subjects did not have more stage 2 sleep. The amplitude criterion of visual scoring (which according to Rechtschaffen and Kales is confined to 75μV for delta waves; Rechtschaffen and Kales, 1968) might play a role in this difference, since older subjects tend to have lower EEG delta wave amplitudes. The significant difference between the age groups in stage 2 was presumably due to the fact that most young volunteers were not able to sleep during the wake maintenance zone. Based on the findings of Steriade et al. (Steriade *et al.*, 1993; for a review see Steriade, 2003), another possible interpretation of our data may be an age-related decrease in the hyperpolarized state of thalamocortical and cortical neurons and thus less synchronization and shorter periods of 'deep sleep' in favor of stage 2. Whether only the electrical potential is dampened with age or the number of neurons firing is reduced remains to be elucidated. A third interpretation for the SWS reduction with age might be a diminished homeostatic drive for sleep in the older group. According to the two process model of sleep regulation (Borbély,

1982; Daan *et al.*, 1984), SWS and SWA depend exclusively on prior duration of wakefulness and exhibit age-related lower absolute levels (Bliwise, 1993), however, a full 'dose-response curve' with different levels of sleep pressure has not been carried out so far with aged subjects. A recent study has found that young and older adults manifest a similar homeostatic response to naps (Campbell and Feinberg, 2005). This is in accordance with previous (Dijk *et al.*, 2000) as well as our data (Münch *et al.*, 2004) looking at the age-related changes in the homeostatic response after sleep deprivation.

Across the 40 hours, the older group showed a shorter mean REM sleep duration, implying a diminished circadian rhythm of REM sleep compared to the young volunteers. Such age differences in mean REM duration have not been found in all studies (Dijk *et al.*, 1999a; Dijk *et al.*, 2001; for a review see Bliwise, 1993), even though a significantly shorter REM duration has been referred from a nap-study with ultra-short sleep-wake cycles (Haimov and Lavie, 1997). Interestingly, mean RL was longer in our aged study group, which is in contrast to a FD study where significantly shorter mean RL for the older subjects was reported (Dijk *et al.*, 1999a; Dijk *et al.*, 2001). Thus, the duration of the imposed sleep-wake cycle may have contributed to this difference.

Subjective sleepiness

OIder volunteers were significantly sleepier during the wake maintenance zone than the young. Whereas higher sleepiness in the older group began already during the first afternoon, it remained low in the young volunteers outside the melatonin secretion phase. Two interpretations are possible: first, the recuperative effect of napping during daytime to decrease homeostatic sleep pressure might be attenuated with age, resulting in higher accumulated sleepiness in the afternoon and evening. Second, the circadian arousal signal in the evening fails to adequately oppose increasing homeostatic sleep pressure in the older group. If the first argument is true, an age-related increase of homeostatic EEG markers during daytime, e.g. an increase of SWA in naps during the biological day and/or theta activity measured in the wake EEG should be observed in the older volunteers. However, there was no

significant difference in SWA between the age groups during naps in the biological daytime. On the other hand, sleepiness and TST during the wake maintenance zone were higher in the older group, which corroborate the second argument. The time course of subjective sleepiness and sleep efficiency (or TST) were not correlated, indicating that subjective sleepiness and the ability to get enough sleep is not implicitly related.

A presumably different impact of the protocol on both age groups should also be taken into account, as spectral analysis of the wake EEG in the young subjects revealed that EEG low frequency components (1-7 Hz) during wakefulness, an index of homeostatic sleep pressure during wakefulness (Cajochen *et al.*, 2001), were slightly enhanced on the second when compared with the first day of the nap protocol (Cajochen *et al.*, 2001). This suggests that even though theoretically sufficient sleep opportunities were presented, the fact that sleep was interrupted during the biological night (3 naps of 75 min) may have led to a short-term enhancement of homeostatic sleep pressure. Clarification awaits the final analysis of EEG low frequency components during wakefulness in both age groups.

Melatonin secretion

Compared to the young volunteers, the mean melatonin secretion in our older group was decreased during the biological night, in accordance with several other studies (Sack *et al.*, 1986; Van Coevorden *et al.*, 1991; Magri *et al.*, 2004, for reviews see Kennaway *et al.*, 1999; Riemersma *et al.*, 2004). The reason for this decline of hormonal secretion with age is unknown and not correlated with the size of the pineal organ (Kunz *et al.*, 1999).

It is well established that melatonin secretion is enormously different between individuals (‘low secretor’ vs. ‘high secretor’), which could be a reason why not all populations studied reveal such age differences (Zeitzer *et al.*, 1999). When young and older subjects of that study with the lowest plasma melatonin values were binned together (e.g. the lower 15 percentile of each group), a significant reduction in the older group could be demonstrated in the 24-h average melatonin secretion and in the average nocturnal peak concentration (Zeitzer *et al.*, 1999). Absolute levels of

melatonin secretion do not correlate with sleep quality in the elderly (Youngstedt *et al.*, 1998) nor does administration of exogenous melatonin unambiguously improve sleep-wake behavior in healthy older people (for a review see Riemersma *et al.*, 2004). On the other hand, there is an established association between the nocturnal 'sleep gate' and the onset of melatonin secretion (Tzischinsky *et al.*, 1993) in younger subjects, shortly before habitual bedtime and immediately after the wake maintenance zone. When exogenous melatonin is administered in the late afternoon, the sleep time of young volunteers is advanced, permitting sleep even during the wake maintenance zone, which supports the tight association between melatonin onset and sleep gating (Rajaratnam *et al.*, 2004). From this, one could argue that changes in the timing of melatonin onset have repercussions on the timing of sleep (Cajochen *et al.*, 2003). Interestingly, the often reported age-related advanced sleep timing relative to circadian phase markers such as melatonin or CBT (Duffy *et al.*, 1998; Youngstedt *et al.*, 2001; Duffy and Czeisler, 2002; Duffy *et al.*, 2002) was not found in our study nor by others (Monk *et al.*, 1995). We found no phase advance in the upward mean crossing time or the midpoint of melatonin secretion, nor in the duration of secretion in the older age group; neither did the average bed- and wake-up times reveal significant differences between the age groups. Moreover, there were no significant age differences in the phase angles (e.g. melatonin upward- and downward mean crossing times since elapsed time awake). The only melatonin parameter which differed significantly was the lower mean melatonin secretion during the biological night (see above). Taken together, the altered age-related change in the sleep-wake pattern was presumably not determined by a phase advance in sleep-wake timing nor in shifts of the circadian phase marker (melatonin) in relation to the timing of sleep and wakefulness (the CBT analyses point in the same direction; unpublished data).

Biological day night differences of the EEG spectra

Significant biological day-night differences between young and older volunteers were mainly found in the lower alpha and in the spindle range. Several studies have previously demonstrated an age-related reduction in EEG spindle activity (Dijk *et al.*,

1989b; Wauquier, 1993; Landolt *et al.*, 1996; Wei *et al.*, 1999; Carrier *et al.*, 2001). The circadian modulation of sleep spindles (Dijk and Czeisler, 1995) and the influence of exogenous melatonin during daytime in enhancing activity in the low spindle frequency range (13.75–14 Hz) and reducing activity in the high spindle frequency range (15.25–16.5 Hz) has been described in young subjects (Dijk *et al.*, 1995). During the biological night (when endogenous melatonin is secreted) the peak in the EEG spindle range is (in young subjects) at a lower frequency range than during the biological day (outside the melatonin secretion window; Dijk *et al.*, 1995; Dijk *et al.*, 1997; Knoblauch *et al.*, 2003). These biological day-night shifts were found in both age groups of our study. However, in the older volunteers the nocturnal peak in the lower spindle frequency range was significantly attenuated and the nocturnal reduction in the higher spindle frequency range was significantly less pronounced, when compared to the young volunteers. The relative spectral differences in the spindle frequency range demonstrate a smaller shift between biological day and night spectra in the older group and were found in all derivations, but most pronounced in Pz. The supposed relationship between an age-related attenuated melatonin secretion and the altered activity in the nocturnal EEG spindle frequency range is not fully understood. The effects of exogenous melatonin on the sleep EEG are reminiscent of those induced by benzodiazepines, which act as $GABA_A$ agonists and enhance spindle generation (particularly in the low spindle frequency range; Borbély *et al.*, 1985) in the nucleus reticularis of the thalamus (Lancel, 1999).

Whether the circadian rhythms of sleep spindles are generated by the SCN directly through neuronal pathways (Novak *et al.*, 2000b) or indirectly via other pathways (or both) is not known. In rodents, direct projections from to the SCN to the thalamus (paraventricular nucleus) with highest neuronal activation during daytime have been recently reported (Novak *et al.*, 2000b). Concomitantly, the SCN may project indirectly via the dorsomedial hypothalamus (DMH) to the ventrolateral preoptic nucleus (VLPO; Chou *et al.*, 2002) with most active neurons during sleep (Sherin *et al.*, 1996), for a review see (Pace-Schott and Hobson, 2002). The VLPO projects via GABAergic neurons to wake-promoting regions such as the histaminergic tuberomammillary nucleus and other monaminergic nuclei and has therefore a sleep-promoting effect (Sherin *et al.*, 1998; Lu *et al.*, 2002).

A negative correlation between neuronal activity of the VLPO and the PVT seems likely to play a role in the regulation of sleep and wakefulness, at least in rodents (Novak *et al.*, 2000a; Novak *et al.*, 2000b). Therefore, the significantly smaller day-night differences in the EEG spindle frequency range as well as in the alpha frequency range of our aged human study group might be due to an age-related attenuation of the circadian signal emanating from the SCN to the DMH and hence the VLPO, with consequently reduced inhibition of the brain stem ascending reticular activating system during biological nighttime. This may further result in higher arousability during sleep with less sensory inhibition of the thalamic nuclei, implying a reduced circadian modulation of sleep and wakefulness in the ageing organism. More detailed analyses of the age-related changes in the EEG spindle range will be reported elsewhere (Knoblauch *et al.*, 2004).

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

Acknowledgements

We thank Claudia Renz, Marie-France Dattler, Giovanni Balestrieri, Dr. Peter Graw and the student shift workers for their excellent help in data acquisition. We also thank all volunteers for their great compliance in a demanding study. This research was supported by Swiss National Foundation Grants START 3100-055385.98 and 3130-054991.98 to CC, the Velux Foundation (Switzerland) and Bühlmann Laboratories, Allschwil (Switzerland).

CHAPTER 4

HOMEOSTATIC SLEEP REGULATION UNDER LOW SLEEP PRESSURE: ARE THERE AGE EFFECTS?

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Manuscript submitted for publication

Summary

We have previously shown that, compared with the young, healthy older volunteers respond to high sleep pressure conditions (i.e. 40 hours of wakefulness) with an attenuated frontal predominance of sleep EEG delta activity. Here, we investigated age-related changes in homeostatic sleep regulation under low sleep pressure conditions. Sleep pressure was kept low by intermittent 75-min naps scheduled every 150 min for 40 hours under constant posture conditions. The EEG was continuously recorded during an 8-h baseline sleep episode during the 40-h multiple nap protocol and during the following 8-h recovery sleep episode in sixteen young (20-31 years) and 15 older (57-74 years) healthy volunteers. In response to sleep satiation we observed a significant decrease of EEG power density in the delta range and a significant increase in the sleep spindle range during recovery sleep, which did not significantly differ between the age groups in the first NREMS episode. However, the relative EEG delta response to sleep satiation lasted significantly longer in the young (across the first 2 NREM episodes) than in the older participants (only the first NREM sleep episode). In contrast to high sleep pressure (frontal predominance), the sleep EEG delta response after low sleep pressure was predominantly occipital in both age groups. Thus, our results suggest that it is the time course of the dynamics of EEG delta power during recovery sleep in response to low sleep pressure that is changed with ageing, rather than a difference in regional sleep aspects. Additionally, the homeostatic sleep response to low sleep pressure is similar in healthy older and young subjects.

Introduction

Human sleep undergoes homeostatic regulation that is functionally expressed by sleep need and sleep propensity (Campbell and Tobler, 1984; Tobler, 2005). With elapsed time awake, the power of low frequency components (<8 Hz) in the human EEG accumulates exponentially and decreases during the following sleep episode (Borbély *et al.*, 1981; Dijk *et al.*, 1987a; Dijk and Czeisler, 1995). These reliable physiological hallmarks reflect a higher synchronized thalamocortical and cortical neuronal firing pattern (Steriade *et al.*, 1993). Sleep homeostasis may be functionally linked with restoration processes, but the underlying mechanisms remain to be elucidated (Benington and Heller, 1995; Krueger and Obal Jr, 2003; Tononi and Cirelli, 2003). There is mounting evidence that genetic factors (Franken *et al.*, 2001; Rétey *et al.*, 2005) and processes related to synaptic plasticity (Tononi and Cirelli, 2003) are involved in slow wave sleep (SWS) and slow-wave activity (SWA; EEG frequency range between 0.75-4.5 Hz) regulation and thus in sleep homeostasis.

Challenging sleep homeostatic processes in humans by prolonged wakefulness (sleep deprivation), has revealed an increase of both EEG theta activity (4.5-8 Hz) during wakefulness (Cajochen *et al.*, 1995; Cajochen *et al.*, 1999b; Cajochen *et al.*, 2001; Finelli *et al.*, 2000) and SWA at the beginning of the recovery sleep episode (RC), predominantly in frontal brain areas (Werth *et al.*, 1997; Cajochen *et al.*, 1999a; Finelli *et al.*, 2001). On the other hand, lowering homeostatic sleep pressure by naps led to a decrease in EEG SWA (Feinberg *et al.*, 1992; Knoblauch *et al.*, 2002) and EEG theta activity (Werth *et al.*, 1996), during the post-nap night (Werth *et al.*, 1996; Knoblauch *et al.*, 2002; Campbell and Feinberg, 2005). In contrast to a frontal predominance of the relative SWA increase after sleep deprivation, we have evidence for an ‘occipital predominance’ of relative SWA reduction after sleep ‘satiation’ (Knoblauch *et al.*, 2002). Taken together, the timing as well as the duration of the scheduled sleep or nap episodes in the above mentioned studies determined the level of SWA during the following sleep episodes (Feinberg *et al.*, 1992; Werth *et al.*, 1996; Feinberg and Campbell, 2003). This is in accordance with the postulated predictive quantitative relationship of the duration of prior wakefulness and the following SWA during sleep (Akerstedt and Gillberg, 1986).

Healthy ageing is known to cause less consolidated sleep (Dijk *et al.*, 1999a), more daytime napping and higher sleepiness during the day (Buysse *et al.*, 1992). Additionally, most studies have reported advanced bed- and wake-up times in healthy elderly (Monk *et al.*, 1995; Carrier *et al.*, 1999), and some of them an altered circadian phase angle between habitual wake time and endogenous circadian phase markers such as melatonin or core body temperature (Duffy *et al.*, 1998; Duffy *et al.*, 2002). Besides an attenuation of circadian amplitude with age, impairment of the homeostatic sleep/wake regulatory system in the elderly has been suggested to contribute to the above mentioned age-related changes (Dijk *et al.*, 1999a). The homeostatic SWA response to sustained wakefulness in older participants is comparable with, but in several details not congruent to that of younger ones. Older subjects very consistently show reduced absolute SWA (and SWS) levels during both BL and RC (Dijk *et al.*, 1989b; Landolt *et al.*, 1996; Carrier *et al.*, 2001) compared to the young. It remains controversial whether this general decrease in power of low EEG components in the elderly reflects an age effect *per se* (e.g. generally altered cortical functions; 'frontal ageing hypothesis'; Horne 1992) or whether an additional, age-related dysfunction of the homeostatic system leads to the above mentioned changes.

Whether the homeostatic response to high sleep pressure is similar in older and young subjects is still not clear. There is evidence from a recent study that the elderly had a significantly lower rebound of SWA during the RC after 25-h of sleep deprivation (Gaudreau *et al.*, 2001b; Drapeau and Carrier, 2004). However, since the sleep deprivation episode in this study ended at the habitual wake time, the circadian change imposed by this protocol precludes direct interpretation of the sleep homeostatic response. The elderly have a higher arousal level at habitual wake time that would diminish their delta response to sleep loss. We have recently found a significant attenuated frontal predominance of EEG delta power during RC in older volunteers in response to 40-h of sleep deprivation ending at habitual bedtime (Münch *et al.*, 2004). Moreover, the dissipation of SWA in the course of the RC (after SD) exhibited a shallower decline in the elderly (Dijk *et al.*, 1999b; Münch *et al.*, 2004).

Rather few experiments under low sleep pressure conditions with older volunteers have been conducted so far (Richardson *et al.*, 1982; Haimov and Lavie,

1997; Monk *et al.*, 2001; Feinberg and Campbell, 2003; Niggemyer *et al.*, 2004; Buysse *et al.*, 2005; Campbell *et al.*, 2005; Münch *et al.*, 2005), and to our knowledge, only one study group has compared the post-nap sleep episode of young and older volunteers (Campbell and Feinberg, 2005). In that report Campbell and Feinberg showed a similar homeostatic sleep response to a daytime nap (repeated at four different times of day), between younger and older volunteers, as indexed by an equally reduced EEG delta (0.3-3 Hz) power during the following RCs in both age groups (Campbell and Feinberg, 2005).

Our first analyses of the 40-h nap protocol indicated that our older volunteers were sleepier in the late afternoon and evening ('wake maintenance zone'; Münch *et al*, 2005) than the young. Furthermore, day/night differences in the lower alpha and spindle range were less pronounced compared to younger subjects, even though the total amount of sleep was not different, nor did wakefulness during sleep episodes differ across naps (for more results see Knoblauch *et al.*, 2005; Münch *et al.*, 2005). Here we focus on the question whether homeostatic sleep regulation shows age-related alterations under low sleep pressure conditions. If sleep homeostasis undergoes a general impairment with age, we hypothesized a weaker response to sleep satiation as indexed by a attenuated decrease in SWA during the RC (relative to the BL) after 40-h of intermittent napping in the older subjects when compared to the young subjects.

Methods

Study participants

Potential study participants were recruited via advertisements in newspapers and at different Swiss Universities. Sixteen young (8 women, 8 men; age range 20-31 years; 25.3±3.3 years; mean±SD), and fifteen older (7 women, 8 men; age range 57-74 years; 65.1±5.6 years; mean±SD) were selected for the study. The screening procedure included detailed questionnaires, a medical examination, and for the older group a neuropsychological assessment (CANTAB® test battery and the Stroop Test) was also carried out to exclude motor, attention, or memory impairments. A screening night was recorded to exclude the presence of sleep disorders. The criteria

were: a sleep efficiency of at least 80%, fewer than 10 periodic leg movements per hour, and an apnea/hypopnoea index lower than 10. Only participants without any medication (with the exception of 4 younger women using oral contraceptives) were included in the study. The drug-free status was verified by urinary toxicological analysis (for the young participants). All study participants were free from medical, psychiatric and sleep disorders, were non-smokers and had no shift work or flights exceeding three time zones during the last three months before study begin. Extreme chronotypes, as assessed by the morningness-eveningness type questionnaire, were excluded (Torsvall and Åkerstedt, 1980). Older volunteers had slightly but significantly higher ratings (earlier chronotypes) than the younger group (18.8±3.0 vs. 16.3±3.3; mean±SD; t-test, $p<0.05$). The Pittsburgh sleep quality index (PSQI, inclusion criterion score ≤ 5; Buysse *et al.*, 1989) was also slightly higher in the older than in the younger subjects (3.4±1.7 vs. 2.1±1.3; mean±SD; t-test, $p<0.05$). Younger female participants were studied during the follicular phase of their menstrual cycle. All subjects were paid to participate and gave their signed informed consent. The study protocol, screening questionnaires and consent form were approved by the local Ethics Committee and conformed to the Declaration of Helsinki.

Protocol

The protocol comprised two baseline sleep episodes in the chronobiology laboratory, followed by a 40-h multiple nap protocol with 10 alternating sleep/wake cycles of 75/150min duration and one recovery sleep episode (RC, Figure 1). Polysomnographic recordings and constant posture started in the afternoon after the first BL. Henceforth, subjects remained in dim light conditions (<8 lux during wakefulness, 0 lux during sleep episodes) under constant semi-recumbent posture position in bed with regular meals and no time cues (for details of the protocol see Cajochen *et al.*, 2001). The older participants received a daily low dose heparin injection (Fragmin®, 0.2ml, 2500 IE/UI, Pharmacia AG, Dübendorf, Switzerland) in order to prevent any venous thrombosis. One week before study begin (baseline week) subjects were asked to abstain from excessive caffeine and alcohol

consumption (at most one caffeine-containing beverage per day and less than five alcoholic beverages per week were allowed).

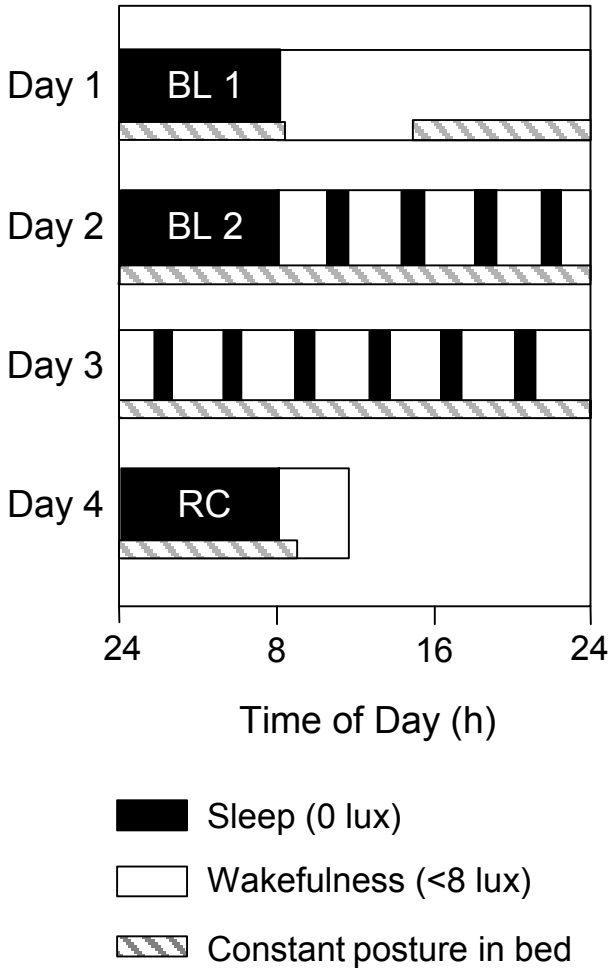

Figure 1

Schematic illustration of the study protocol with two 8-h baseline sleep episodes (BL), ten 75-min naps and one 8-h recovery sleep episode (RC). Black bars (0 lux) indicate scheduled sleep episodes and white bars episodes of scheduled wakefulness (<8 lux). Hatched bars delineate the time of controlled posture position (semi-recumbent during wakefulness and supine during sleep).

They were instructed to keep a regular sleep-wake schedule during the baseline week at home (i.e. bed- and wake times within ± 30 min of a self-selected target time between 10:00 pm and 2:00 am) prior to admission to the laboratory. Compliance was verified by sleep logs and ambulatory activity measurements (wrist activity monitor, Cambridge Neurotechnologies®). The timing of the seep-wake schedule during the protocol was adjusted to individual habitual bedtimes. For each subject, habitual bedtime was calculated by centering the approximately 8-h sleep episodes during the baseline week at their midpoint. Habitual bedtimes did not significantly differ between the age groups: 11:39 pm±52 min (younger) vs. 11:11 pm±40 min (older; mean±SD; t-test: p=0.1).

Sleep recordings and data analysis

Sleep episodes were polysomnographically recorded using the Vitaport Ambulatory system (Vitaport-3 digital recorder TEMEC Instruments BV, Kerkrade, the Netherlands). Twelve EEG derivations (F3, F4, Fz, C3, C4, Cz, P3, P4, Pz, O1, O2, Oz), two electrooculograms, one submental electromyogram and one electrocardiogram were registered, referenced against linked mastoids and online digitized (12 bit AD converter, 610μV/bit) with a storage sampling rate at 128 Hz for the EEGs. All signals were digitally filtered at 30 Hz (fourth order Bessel-type anti-aliasing filters, total 24 Db/Oct.) with a time constant of 1 s. The raw signals were stored online on a PC card and downloaded offline to a PC hard drive. EEG artefacts were detected by automated artefact detection software (CASA, 2000 Phy Vision BV Kerkrade, The Netherlands). All sleep episodes were visually scored according to standard criteria (Rechtschaffen and Kales, 1968) per 20-s epoch. EEGs were subjected to spectral analysis using a fast Fourier Transform (FFT, 10% cosine 4-s window), which resulted in a 0.25 Hz resolution. Artefact-free 4-s epochs were averaged over 20-s epochs and matched with the 20-s epochs of the sleep scoring.

Sleep stages were expressed as percentage of total sleep time [TST: (∑ stages 1-4, REM sleep)]. TST and sleep latencies (SL) and REM sleep latency (RL) were indicated in minutes and sleep efficiency (SE) as percentage of TST per

scheduled sleep time (SE=TST/time between lights off and lights on x 100). WALO indicates wakefulness after lights off and WASO wakefulness after sleep onset.

The EEG spectra during the BL and RC were calculated in the frequency range from 0.5 to 25 Hz for the midline derivations (Fz, Cz, Pz and Oz) on log-transformed data. The RCs were expressed as percentage of BL mean. For graphical illustration, the log-transformed mean values per subject were averaged for each age group separately and then re-transformed. Gender differences on EEG power density were assessed by repeated measures ANOVAs, which yielded a significant three-way interaction ('gender' x 'age' x 'derivation') for relative EEG power density in the frequency range between 0.5-1 Hz (3-way rANOVA; $F_{3,81}>2.8$, $p<0.05$). However, *post-hoc* comparisons between men and women for the different EEG derivations and age groups revealed no significant gender differences in this frequency range.

NREM/REM sleep cycles were defined according to the criteria of Feinberg and Floyd (Feinberg and Floyd, 1979), with the exception that for the last sleep cycle, no minimum REM sleep duration was required. Thereafter, each sleep cycle was divided into ten equal time intervals during NREM sleep and four equal time intervals during REM sleep.

In order to compare the sleep EEG (during NREM sleep) in the absolute SWA range during the first two 30-min intervals of BL, naps and RC, nap 4 and 10 were excluded from the analyses, because not enough participants, particularly the young, were able to sleep during these two naps, which were scheduled during the wake maintenance zone in the late evening (Knoblauch *et al.*, 2003; Knoblauch *et al.*, 2005; Münch *et al.*, 2005). Furthermore, only participants able to sleep (i.e. assessed by the first occurrence of stage 1) during all remaining naps and the first 60 min of BL and RC were included in this analysis (8 young and 11 older participants).

Statistics

The statistical packages SAS® (SAS Institute, Inc., Cary, NC; Version 6.12) and Statistica® (StatSoft Inc., STATISTICA for windows, Tulsa, OK, USA, Version 6.1) were used. EEG power density was averaged during NREM sleep and expressed as

log-ratio (RC/BL) per subject. One-, 2- and 3-way repeated measures ANOVAs (rANOVA) were used with the categorical factor 'age group' (young vs. older) and the repetitive factors: 'derivation', 'time interval' or 'sleep cycle'. All *p*-values derived from rANOVAs were based on Huynh-Feldt corrected degrees of freedom, but the original degrees of freedom are reported. For *post-hoc* comparisons, Duncan's multiple range test and t-tests were performed. If normal distribution was lacking, non-parametric tests were applied (Mann-Whitney U: for sleep stages during the naps; and Wilcoxon matched-pairs test: for sleep stages within each age group, when compared to BL). *Post-hoc* comparisons were corrected for multiple comparisons according to Curran-Everett (Curran-Everett, 2000).

To investigate the decay of EEG delta power density (0.5-1.25 Hz) across the BL and the RC night between young and older volunteers, an exponential decay function was fitted to the data of all subjects and NREM cycles: $delta=delta_0 \times e^{(-rt)}$; with $delta_0$ =intercept of the y-axis, delta=mean EEG delta power per sleep cycle, r=slope of the decay, t=average timing of the cycle midpoint.

Results

Sleep Stages

RC versus BL sleep episode

Visually scored sleep stages during the BL and RC between young and older volunteers are summarized in Table 1. The older participants had significantly less TST, stage 3, 4, SWS, and a lower SE than the young in both the BL and RC ($p<0.05$; $F_{1,29}$ at least 4.1; two-way rANOVA: main effect of age group). Furthermore, older subjects were significantly longer awake after lights off (WALO) and after sleep onset (WASO) and had more stage 2 ($p<0.05$; $F_{1,29}$ at least 9.3; main effect of age group) during both nights. The factor 'night' yielded significance for TST, SL, SE, WALO, WASO, MT, stage 1 and 4 ($p<0.05$; $F_{1,29}$ at least 4.6; two-way rANOVA) with a tendency for SWS ($p<0.1$). The interaction between the factors 'age group' and 'night' yielded significance for stage 2, 4 and SWS ($p<0.05$; $F_{1,29}$ at least 5.3; two-way rANOVA). This interaction came about via reduction of stage 2 during the RC in comparison to BL in the older subjects, while the amount of stage 2 in RC remained

unchanged in the young (p<0.05; Duncan's multiple range test). In the case of SWS, the young participants responded with less SWS in the RC to the multiple nap protocol, while the older participants showed no changes in SWS in response to the multiple nap protocol (p<0.05; Duncan's multiple range test).

Comparisons between BL and RC within each age group separately resulted in more WALO, longer sleep latencies to stage 1 (SL1) and 2 (SL2), less TST and lower SE during the RC for both age groups ($p \leq 0.05$; $F_{1,14}$= at least 5.3 for the older and $F_{1,15}$= at least 5.5 for the young). Only for the older participants was a significant increase in MT (p<0.05; $F_{1,14}$=5.6; one-way rANOVA) during RC present, and only for the young subjects did we find significantly less stage 4 and SWS during RC compared to BL (p<0.05; $F_{1,15}$= at least 6.7; one-way rANOVA).

Nap versus BL sleep episode

Each sleep stage (% of TST) was summed up over the 10 naps (12.5 hours) and compared to the respective mean values during the BL night (8 hours), for each subject and age group separately (data not shown). There was no significant difference between the BL and the naps in the young and the older volunteers for stages 3, 4 and SWS (for all: Wilcoxon matched-pairs test, p>0.1). Both age groups showed significantly less stage 1, shorter SL1 and SL2 during BL and concomitantly more REM, NREM sleep and a higher SE (p<0.05) in comparison to nap sleep. Only the young group had more stage 2 and a longer RL during the BL, while the elderly slept significantly less (TST) and were longer awake (wakefulness + movement time) during the BL than during the naps. Finally, TST during the nap protocol did not significantly differ between the age groups (across 10 naps; Mann-Whitney U test; p<0.05). For detailed information concerning age-related changes in sleep across the 40-h nap protocol, see (Münch *et al.*, 2005).

Table 1

Sleep stages derived from visual scoring for both age groups, averaged across BL and RC, n=16 for young and n=15 for older subjects (mean±SEM).

	Baseline		**Recovery**							
	Young	**Older**	**Young**	**Older**						
					age $F_{1,29}$	*p*	night $F_{1,29}$	*p*	age x night $F_{1,29}$	*p*
TST	441.4±6.3	408.9±12.0	413.7±11.8	368.7±14.0	8.1	*	16.9	**	0.6	
SE (%)	92.0±1.3	85.2±2.5	86.2±2.5	76.9±2.9	8.1	*	16.9	**	0.5	
WALO (%)	6.1±1.4	16.8±3.5	13.7±3.4	29.2±5.6	9.35	*	10.7	*	0.7	
WASO (%)	4.0±1.2	14.5±3.2	8.3±2.3	22.9±5.2	11.1	*	5.7	*	0.6	
MT (%)	2.9±0.4	2.2±0.3	4.0±0.8	4.0±0.7	0.27		11.1	*	0.6	
Stage 1 (%)	12.3±1.2	14.1±1.3	14.4±1.2	15.6±1.4	0.9		4.8	*	0.1	
Stage 2 (%)	**49.7±1.2**	**60.8±2.4**	**51.3±1.5**	**57.4±2.6**	**10.9**	*	**0.7**		**5.3**	*
Stage 3 (%)	10.2±1.0	6.7±1.2	9.4±0.7	7.0±1.1	4.6	*	0.3		2.0	
Stage 4 (%)	**7.5±1.3**	**1.5±0.5**	**5.5±1.1**	**1.6±0.6**	**14.9**	**	**4.6**	*	**5.3**	*
SWS (%)	**17.7±1.6**	**8.2±1.7**	**14.8±1.4**	**8.6±1.6**	**13.3**	*	**3.7**	°	**7.0**	*
NREM (%)	67.4±1.3	69.0±1.9	66.1±1.0	66.0±1.7	2.2		0.2		1.3	
REM (%)	20.3±1.1	16.9±1.3	20.0±1.2	18.4±1.4	2.2		0.2		1.3	
SL1 (Min)	9.3±1.3	8.6±1.6	20.2±5.0	22.5±3.6	0.3		27.9	**	2.7	
SL2 (Min)	14.7±1.9	12.1±2.1	29.7±5.0	33.3±6.4	0.1		57.5	**	2.4	
RL (Min)	68.9±1.1	80.1±9.9	80.8±10.5	94.4±8.5	1.7		1.5		0.6	

TST=total sleep time (min; stages 1-4 + REM sleep); SE=sleep efficiency [%; (TST/time between lights off and lights on) x 100]; WALO=%; (wakefulness after lights off/TST x 100); WASO=% of wakefulness after sleep onset [(wakefulness -SL1)/TST x 100]; MT=movement time (%; movement time /TST x 100); Stage 1-4=% of TST; SWS=slow wave sleep (% of TST; stage 3 + 4); NREM sleep =Non-rapid eye movement sleep (% of TST; stage 2 - 4); SL1 (min) =sleep latency to stage 1; SL2 (min) =sleep latency to stage 2; RL (min) =latency to REM sleep (SL1, SL2 and RL on log-transformed values). Column 6 indicates main effect of 'age group' (two-way rANOVA) and column 7 depicts the main effect of 'night'. The last column shows the interaction between the factors 'age group' x 'night'. All F-values are indicated; *=$p<0.05$, **=$p\leq0.001$, °=$p<01$, and significant 3-way interactions are shown in bold.

Relative EEG spectra

BL vs. RC sleep episode

Young participants showed significantly less relative EEG power (% of BL) during the RC than the older in some of the frequency bins in the delta (0.5-1.25 Hz) and significantly more in some of the frequency bins in the theta (7.25-7.75 Hz) range (Figure 2; two way rANOVA, main effect of age group; $p<0.05$). The interaction for the factors 'age group' x 'derivation' yielded significance in the delta frequency bins (0.5-1 Hz, 1.75-2 Hz, 3-3.5 Hz) and in the alpha frequency bins (10-10.5 Hz, 10.75-11.25 Hz; $p<0.05$). Post-hoc comparisons resulted in significant lower values for younger subjects during the RC in the delta bins for Cz (0.5-1 Hz), Pz (0.75-1 Hz) and Oz, (0.5-1 Hz, 1.75-2 Hz, 3-3.5 Hz; black squares in Figure 2 represent these *post-hoc* comparisons), and concomitantly higher values in the alpha range for Oz (10-10.5 Hz) than older participants ($p<0.05$; t-test and corrected for multiple comparisons; Curran-Everett, 2000). Finally, the main factor derivation was significant in some frequency bins in the delta (0.5-1 Hz, 1.75-2 Hz), theta (4.25-7.75 Hz), alpha (8-8.5 Hz, 11.25-12 Hz) and sigma range (12.00-12.75 Hz, 14.75-15 Hz; $p<0.05$).

In a next step, relative EEG spectra per NREM-REM sleep cycle (% of BL cycle) were calculated for both age groups for the midline derivations (Figure 3). Since not all subjects completed four sleep cycles, the analyses were restricted to the first three cycles. A three-way rANOVA with the factors 'age group', 'derivation' and 'sleep cycle' did reach significance for three frequency bins: 1.25-1.5 Hz, 9.5-9.75 Hz and 10-10.25 Hz ($p<0.05$). Therefore, each sleep cycle was analyzed separately (Figure 3). The main effect of 'age group' was significant during NREM sleep episode 1 for frequency bins in the sigma range (15.25-15-5 Hz, 16.5-16.75 Hz) and for NREM sleep episode 2 in the delta range (0.5-1.25 Hz), alpha (8.25-9.75 Hz, 10.5-10.75 Hz)

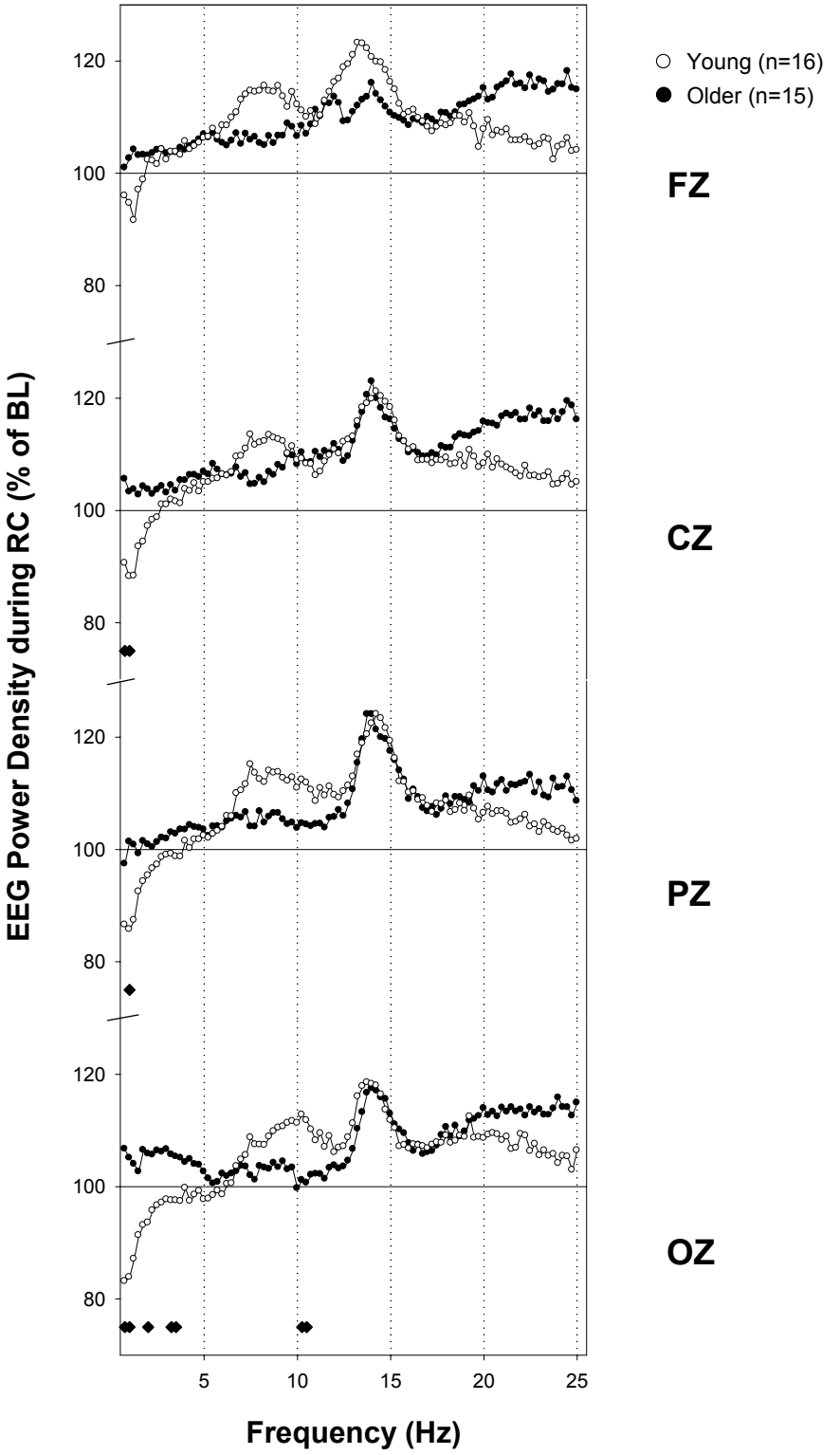

Figure 2

Relative EEG spectra during RC (percentage of BL) between 0.5-25 Hz for Fz, Cz, Pz, and Oz for young (open circles; n=16) and older participants (black circles; n=15). Black diamonds illustrate significant *post-hoc* comparisons between the young and older age group (p<0.05; t-test corrected for multiple comparisons).

and sigma range (11-13.25 Hz, 14.25-15.5 Hz). For NREM sleep episode 3, some frequency bins in the theta (4.25-4.5 Hz, 4.75-5 Hz, 5.25-5.75 Hz, 6-7.75 Hz), alpha (9-9.25 Hz) and sigma frequency range yielded significance (15.5-15.75 Hz; $p<0.05$; two-way rANOVA). The interaction with the factors 'age group' x 'derivation' yielded significance for some frequency bins in the alpha range (9.75-11 Hz, 10.25-10.5 Hz) for cycle 2 and 3 ($p<0.05$), and post-hoc comparisons revealed significantly higher values for the younger volunteers in Pz and Oz during the second and for Oz during the third sleep cycle ($p<0.05$; t-test for independent samples).

To further investigate the time course of EEG delta activity in more detail, percentiles (see methods section) in the frequency range between 0.5-1.25 Hz were expressed as percentage of the BL mean and calculated for NREM-REM sleep cycle 1-3 in Fz, Cz, Pz and Oz (Figure 4). The frequency range of these EEG bands was chosen based on the significant main effect of age group across the relative RC (% of BL) night in this frequency range. A four-way rANOVA ('age group', 'cycle', 'night' and 'derivation') was not significant ($p=0.2$), and therefore each derivation was analyzed separately. A three-way rANOVA ('age group' x 'cycle' x 'night') per derivation revealed significant interaction for Fz, Cz, Pz and Oz ($p<0.05$; F at least 3.3). This interaction most likely reflects the age-related difference in the time course of the 0.5-1.25 Hz band across the first three NREM-REM sleep cycles. *Post-hoc* comparisons within each age group separately (Figure 4) indicated for young and older participants a significant decrease in EEG delta power during the first NREM sleep episode in the RC compared to BL for all derivations along the midline. This significant decrease in EEG delta power continued during the second NREM sleep episode in the young (Cz, Pz; $p<0.05$; tendency for Fz and Oz $p<0.1$; Duncan's multiple range test), but not in the older participants. Comparisons between young and older participants revealed significant higher values for the elderly in the BL during the third sleep cycle (for Fz, Cz, Pz and Oz; $p<0.05$; Duncan's multiple range test), while this significant age-related increase in the RC sleep episode occurred already during the second sleep cycle (for Cz, Pz and Oz; $p<0.05$; Duncan's multiple range test; with a tendency in Fz $p<0.1$).

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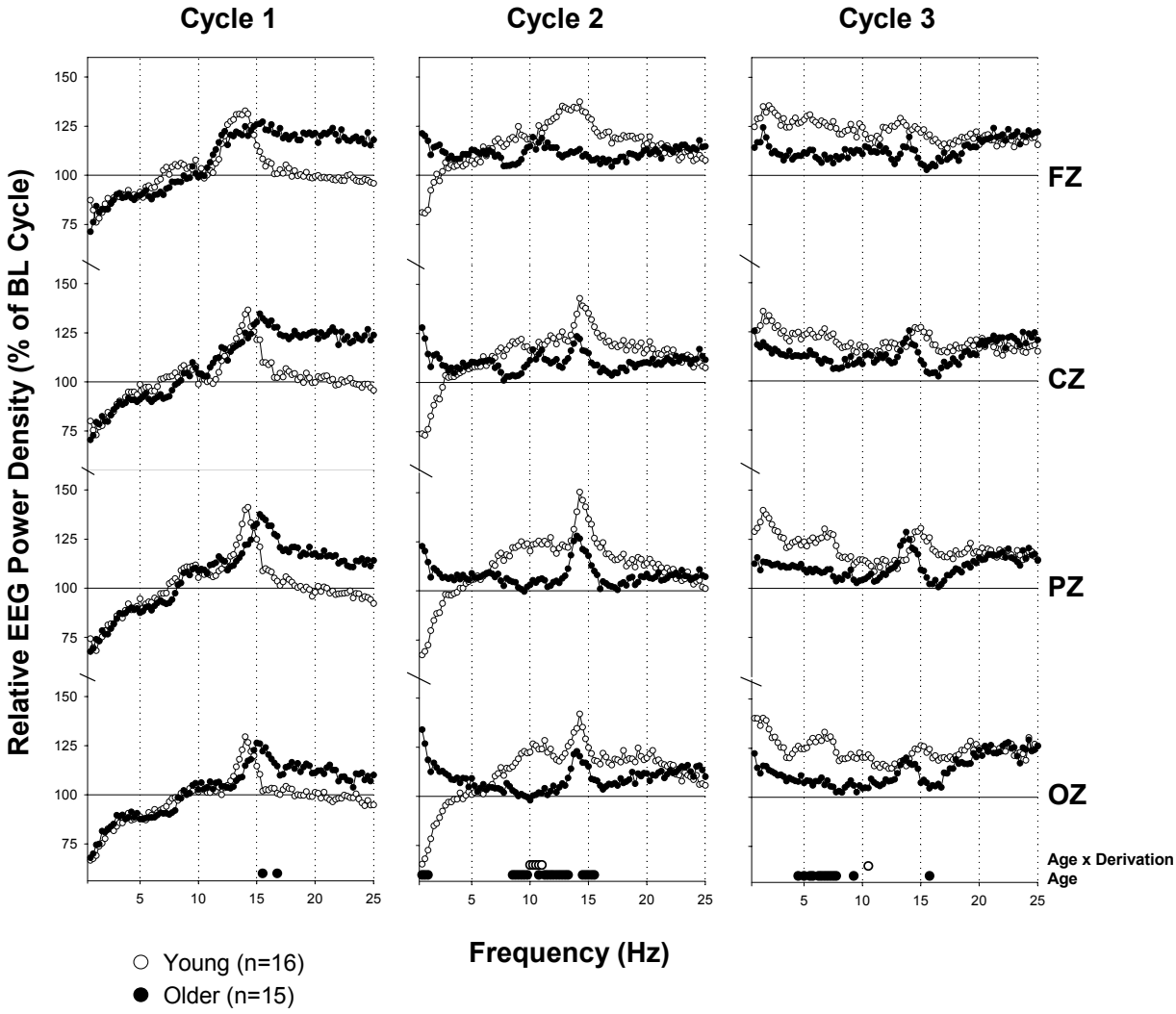

Figure 3

EEG power spectra (percentage of BL cycle) per NREM-REM sleep cycle 1-3 between 0.5-25 Hz for Fz, Cz, Pz and Oz young (open circles; n=16) and older participants (black circles; n=15). Black circles at the bottom indicate significant frequency bins for which the factor 'age' yielded significance, white circles indicate those frequency bins for which the interaction 'age' x 'derivation' yielded significance (two-way rANOVA per sleep cycle; $p<0.05$).

In order to analyze the decay function of EEG delta power for young and older subjects during both nights, we fitted the mean value of all sleep cycles in the relative EEG delta range (0.5-1.25 Hz; % of BL) of all sleep cycles with a nonlinear regression function (see methods). Figure 5 shows the fitted decline of both age groups during the BL and RC for Fz (one data point of the young group is missing on Figure 5, because it was more than 2 SDs away from the next). Table 2 depicts the values of the decay rates for both groups. The mean values of the BL slopes did not overlap with the 95% confidence interval of RC nights within young and older subjects; neither did the mean decay of the BL of one group reach the 95% confidence interval of the BL of the other age group. A slight overlap occurred between the mean RC values of the older with the 95% confidence interval of the RC in the young group (Figure 5, Table 2). Interestingly, the estimated mean decay of the RC night of the young did overlap with the 95% confidence interval of the BL decay values of the older group (the goodness of fit is indexed by R-values for the respective regression curve). The estimation of the delta decay rates during the RC performed for Cz, Pz and Oz revealed R-values <0.5 for both age groups (data not shown).

SWA during BL and RC vs. SWA during naps

In a last step, the build up of SWA during the first 60 min of the BL night, the following 8 nap episodes (without nap 4 and 10, see methods) and the RC was analysed (Figure 6). Mean values of absolute SWA during the first and the second 30 min interval were calculated for both age groups in Fz (during NREM stage1-4, after lights off). Significant higher values were found in the younger group for the first 30-min interval during nap 5, and during the second 30-min interval during the BL and nap 1, 5 and 9 [p<0.05; and tended to be higher in nap 3 and 7 (p<0.1); Mann-Whitney U test].

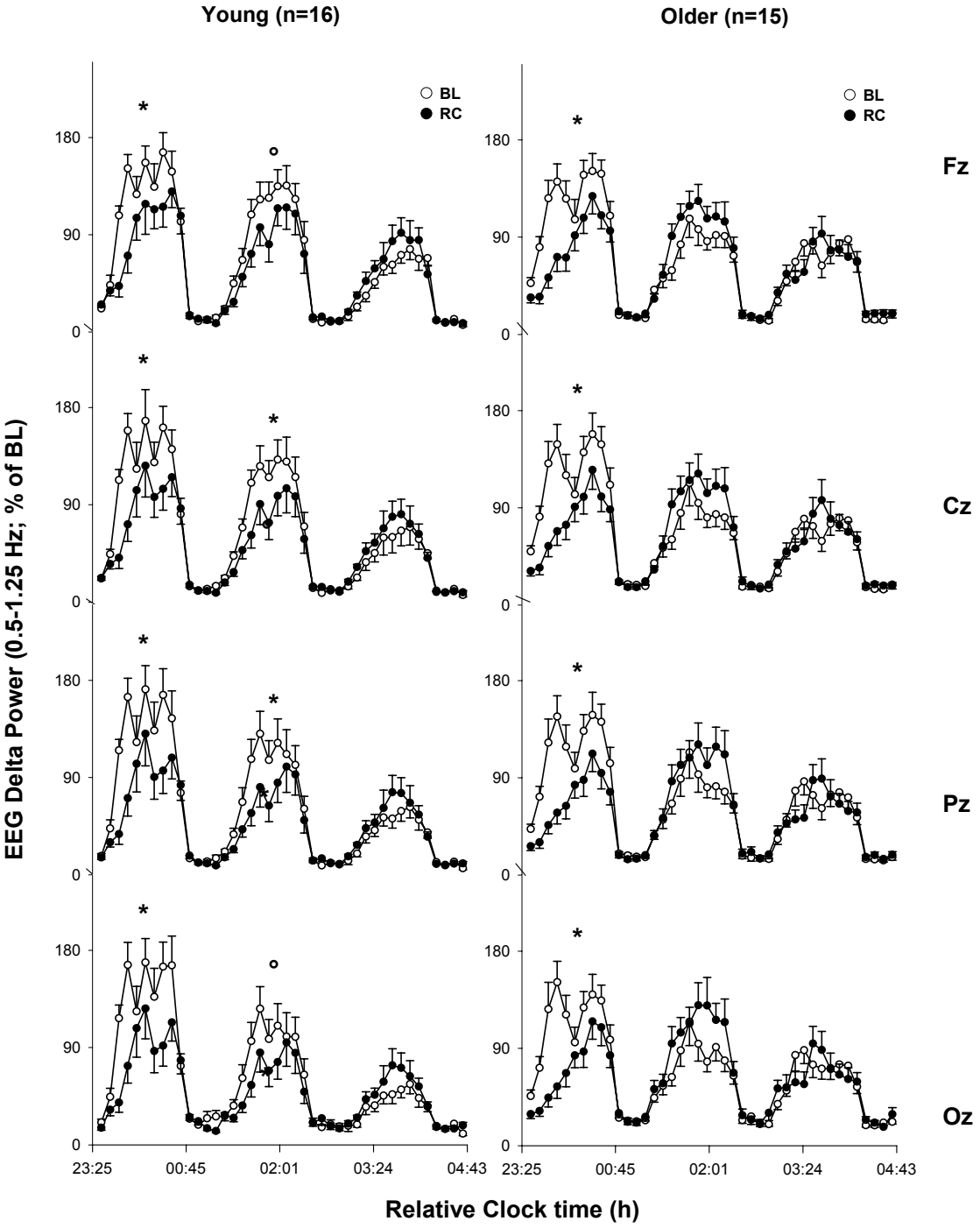

Figure 4

EEG delta activity (0.5-1.25 Hz) per sleep cycle (1-3) for midline derivations (Fz, Cz, Pz and Oz). The left hand panels shows mean values per NREM-REM sleep cycle for the young (n=16) and the right hand panels those for the older age group (n=15; mean + or - SEM). White circles indicate the BL and filled circles the RC. Asterisks indicate significant differences between mean values per cycle between BL and RC within each group (* =p<0.05; ° =p<0.1; two-way rANOVA; 'age' x 'cycle').

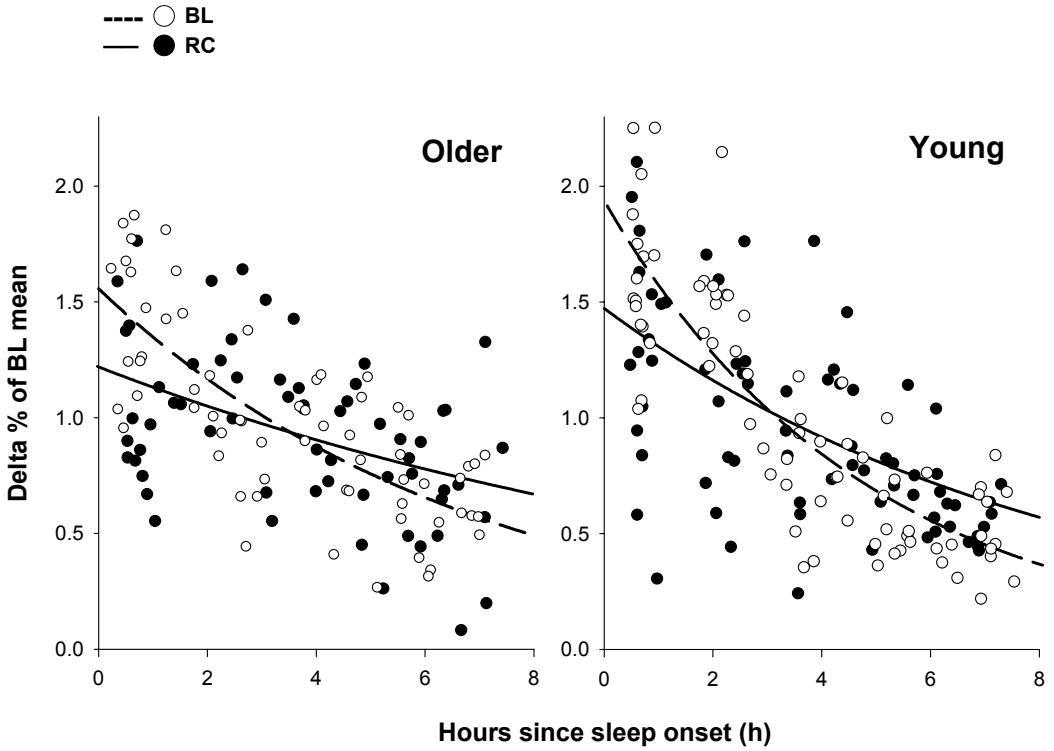

Figure 5

Fitted exponential decay function (delta=delta$_0$ x $e^{(-rt)}$) to relative EEG delta power (0.5-1.25 Hz; percentage of BL) during NREM sleep across all NREM sleep episodes for Fz. Left hand panel: older (n=15), right hand panel: young participants (n=16). Open circles and dashed lines indicate BL values and filled circles together with solid lines the values for the nonlinear regression during the RC (one data point of a younger participant during BL is not shown, was denoted as an outlier, see results).

Table 2

Parameters of the non-linear regression analysis for the decay of EEG delta power during BL and RC for both age groups are indicated for Fz during all NREM-sleep episodes (mean of all cycles ± SEM).

Age group	Night	Estimated Decay Rate (mean per hour ±SEM)	95% Confidence Interval	R-value	$p<0.05$
Young	BL	0.209±0.018	0.174-0.244	0.85	* **
	RC	0.119±0.024	0.072-0.166	0.53	* **
Older	BL	0.144±0.017	0.111-0.177	0.75	* **
	RC	0.074±0.02	0.035-0.113	0.44	*

The 95% confidence intervals are shown for both age groups and nights; * indicates the lack of overlap of mean values with the 95% confidence intervals between BL and RC night within and ** during the BL and RC night between young and older volunteers ($p<0.05$).

To further assess the influence of accumulated sleep pressure on the amount of SWA during the RC, we correlated summed TST across 10 naps (as % of BL) with the relative SWA (% of BL) during the RC for young and older volunteers. From visual inspection, there was a negative correlation between these measures for both age groups (R=-0.2; $p>0.2$; data not shown). The correlation, however, did not reach significance for the young (correlation coefficient R=-0.2; $p>0.3$) but showed a tendency for the older subjects (correlation coefficient R=-0.5; $p=0.07$). Correlation between SWA in the nap and SWA in the RC (both expressed as log-ratio to BL) did not show any significant correlation (data not shown).

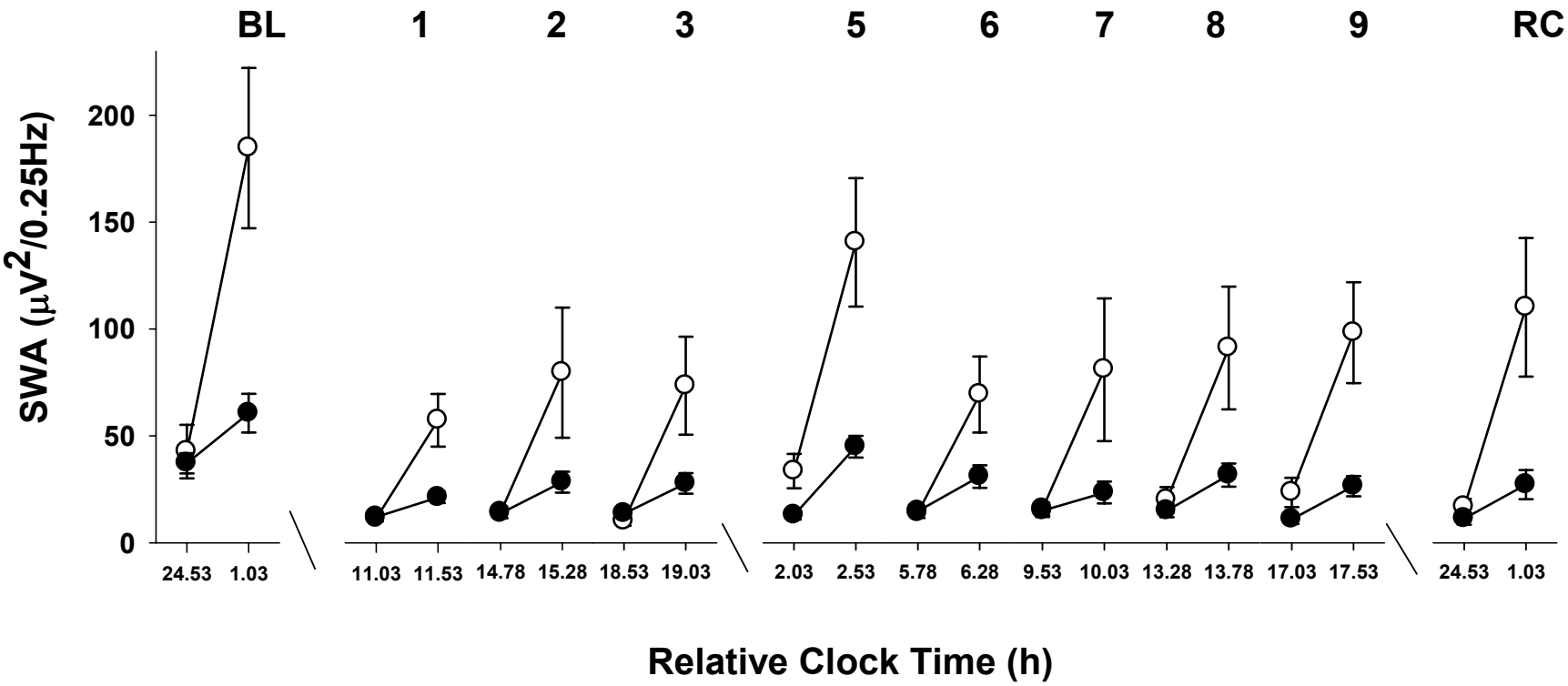

Figure 6

Absolute SWA during the first two 30-min intervals after lights off for Fz. Averaged values are shown during NREM (1-4) for BL, 8 naps (without nap 4, 10) and the RC. Open circles indicate the young (n=8) and filled circles the older subjects (n=11; mean±SEM).

Discussion

We investigated the impact of low sleep pressure on sleep structure and spectral components of the sleep EEG in young and older volunteers. The main differences in the spectral EEG components between both age groups across the entire RC night occurred in EEG frequency bins of the delta and alpha range: the young responded with a significantly stronger relative decline of EEG delta power than the older subjects, predominantly in more occipital brain regions. The 'occipital predominance' of the SWA decline in response to low sleep pressure found in the younger subjects, was not present in the older group. This came about due to an altered time course of EEG delta activity during the RC night, such that during the first NREM cycle EEG delta power declined equally in both age groups, but increased significantly earlier (e.g. during the second sleep cycle) in the older participants. Thus, our results were more complex than originally hypothesized, and did not unambiguously show a weaker homeostatic EEG delta response during the RC in the elderly, when compared to the young. This suggests that low sleep pressure elicits age-related changes in the sleep EEG during the RC night different from those after high sleep pressure (Münch *et al.*, 2004), which is at least true for regional differences (attenuated frontal vs. occipital predominance of EEG delta activity) and the time course of EEG delta activity across the night.

Indices for an age-related weakening of the homeostatic process

Based on two outcomes of this study one could argue for a weaker homeostatic sleep regulation with age: First, the less pronounced EEG delta response to low sleep pressure as observed in the all-night EEG spectra in the older group, and second, the earlier increase of EEG delta power in the course of the RC. Since the amount of prior wakefulness is the most important predictor for sleep EEG delta activity, it could be that our older participants were longer awake during the multiple nap protocol and hence showed a different delta response than the young. This argument can be rejected, because we did not observe any significant differences in TST and SE between both age groups across the precedent ten nap episodes,

although the distribution of TST across the scheduled naps was different between the age groups (Münch *et al.*, 2005). Moreover, the correlation between accumulated TST during the naps and SWA during the post-nap RC tended to be negative for the older group, indicating a typical delta homeostatic response to prior TST or wakefulness respectively. Thus, the overall SWA response to low sleep pressure was adequate in the older group. Nevertheless, it remains to be elucidated if there is objective evidence for higher sleep pressure during the preceding wake episodes. Further clarification is awaited from the waking-EEG analysis. When looking at a subjective marker for sleep pressure during wakefulness, we found that our older volunteers were sleepier than the young in the afternoon and early evening on the first and also on the second day of the nap protocol (Münch *et al.*, 2005). Concomitantly, the elderly performed worse during the biological day (i.e. outside the melatonin secretory phase), as assessed by the Psychomotor Vigilance Test (PVT; Blatter *et al.*, 2006). Therefore, one could argue that the elderly exhibit a faster wake-dependent homeostatic increase of sleep pressure, at least under ‘normal‘ sleep pressure conditions. Another explanation could be that the circadian signal is attenuated in the elderly and thus weaker in opposing the homeostatic sleep pressure build-up during wakefulness. This eventually leads to a more ‘linear’ increase in sleepiness and a not so consolidated 16-h bout of wakefulness as normally seen in young subjects. In line with a weaker circadian arousal signal are the less pronounced day-night differences in the lower EEG alpha and spindle range during the nap sleep episodes (Münch *et al.*, 2005), as well as a weaker coupling of the circadian rhythm of EEG spindle frequency and sleep propensity to the circadian rhythm of melatonin secretion in the older volunteers (Knoblauch *et al.*, 2005). Furthermore, we have evidence for an age-related diminution in the circadian regulation of salivary melatonin (Knoblauch *et al.*, 2005; Münch *et al.*, 2005) and core body temperature (unpublished data). Thus, we argue that rather the interaction of the circadian with the homeostatic process plays an important role in age-related changes of sleep regulation and sleep timing, rather than alterations in the homeostatic process *per se*.

How alterations in the dynamics of EEG delta activity under low sleep pressure are also reflected in cellular and molecular mechanisms is not known. According to Saper et al. (Saper *et al.*, 2005a; Saper *et al.*, 2005b), there is some

evidence that at least one mechanism for sleep drive is the accumulation of a sleep-promoting substance that enhances the activity of sleep-promoting cells and reduces the activity of wake-promoting neurons. Potential mechanisms for a weakened homeostatic sleep regulation with age might be found in the connection between sleep drive, SWA and adenosine concentrations in the forebrain (Benington *et al.*, 1995). Recently, a reduced sensitivity for adenosine (Murillo-Rodriguez *et al.*, 2004) and a decline in adenosine A1 receptors (Meerlo *et al.*, 2004) were found in aged rat brains, which may for the first time indicate a weaker responsiveness of the homeostatic system with age. It remains to be elucidated whether this also plays an important role in the human central nervous system.

Evidence for intact homeostatic sleep regulation in elderly

Both age groups responded to low sleep pressure with lower SE (see above), longer SL's and more WALO during the RC night. Concerning the EEG spectra, which allow quantification of the sleep homeostatic process, a very similar EEG delta power decline during the first NREM cycle was found in both age groups during the RC night. If we assume that the SWA level at the beginning of the sleep episode is one of the most reliable physiological markers for accumulated homeostatic sleep pressure (Borbély, 2001), we do not have strong evidence for an age difference in homeostatic delta response under low sleep pressure, similar to the findings of Campbell and Feinberg (Campbell and Feinberg, 2005). In this study, neither age-related differences in the mean EEG delta response (0.3-3.0 Hz) during the post-nap night were found, nor a change in the period-amplitude incidence of EEG delta power (Campbell and Feinberg, 2005). Furthermore, the increase in EEG power density in the sleep spindle range in response to low sleep pressure was very similar for both age groups in our study, not only at the beginning of the night but also for the remainder of the nocturnal sleep episode. This is a further argument for intact homeostatic sleep regulation in the elderly, since EEG power density in the spindle range is under clear sleep-wake dependent homeostatic control (Knoblauch et al. 2002).

The time constants of the fitted decay function for delta dissipation during BL were different between the age groups, which is in accordance with other studies (Dijk *et al.*, 1989b; Landolt *et al.*, 1996; Carrier *et al.*, 2001). The same was true for the RC night, although the goodness of fit, as indexed by the R-values, was rather low for both age groups, indicating that fitting exponential decay functions is not a very reliable way to fit the delta dissipation after low sleep pressure conditions.

The relationship of this shorter-lived EEG delta response in the elderly to homeostatic sleep regulation requires analysis from the EEG during scheduled wake episodes.

Conclusion

Our results confirm and further extend the conclusion that age-related sleep deteriorations cannot unambiguously be attributed to a weakening in the homeostatic sleep regulatory system. The age-related, diminished occipital decline of EEG delta activity in response to low sleep pressure in the all-night EEG spectra was no longer present when we took a closer look at the time course of the delta dissipation across sleep cycles, where both age-groups showed a similar decrease in EEG delta activity during the first sleep cycle. Subsequently, older participants exhibited an earlier intra-sleep increase of EEG delta activity. Whether this reflects a homeostatic intra-sleep rebound in the elderly, and a possible relationship to the age-related lower sleep consolidation and weakening of homeostatic sleep regulation, remains to be elucidated. We conclude that it is rather the interaction of circadian with homeostatic processes that play an important role in age-related changes in sleep regulation and sleep timing, than alterations in the homeostatic process *per se*.

Acknowledgements

We thank our technicians Claudia Renz, Marie-France Dattler and Giovanni Balestrieri for their excellent help in data acquisition, Drs Carmen Schröder and Corina Schnitzler-Sack for medical screenings, and the volunteers for participating. This research was supported by Swiss National Foundation Grants START 3100-055385.98 and 3130-054991.98 to CC, the Velux Foundation (Switzerland) and Bühlmann Laboratories, Allschwil (Switzerland).

CHAPTER 5

WAVELENGTH-DEPENDENT EFFECTS OF EVENING LIGHT EXPOSURE ON SLEEP ARCHITECTURE AND SLEEP EEG POWER DENSITY IN MEN

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Published in: American Journal of Physiology: Regulatory, Integrative and Comparative Physiology (2006), 290: R1421-1428

Summary

Light strongly influences the circadian timing system in humans via non-image-forming photoreceptors in the retinal ganglion cells. Their spectral sensitivity is highest in the short wavelength range of the visible light spectrum as demonstrated by melatonin suppression, circadian phase shifting, acute physiological responses and subjective alertness. Here we tested the impact of short wavelength light (460 nm) on sleep EEG power spectra and sleep architecture. We hypothesized that its acute action on sleep is of similar magnitude as the reported effects for polychromatic light at higher intensities, and significantly stronger than longer wavelength light (550 nm). The sleep EEG of 8 young men was analyzed after a 2-h evening exposure to blue (460 nm) and green (550 nm) light of equal photon densities (2.8 x 10^{13} photons/cm^2/s) and to dark (0 lux) under constant posture conditions. The time course of EEG slow-wave activity (SWA; 0.75-4.5 Hz) across sleep cycles after blue light at 460 nm was changed such that SWA was slightly reduced in the first and significantly increased during the third sleep cycle in parietal and occipital brain regions. Moreover, blue light significantly shortened REM sleep duration during these two sleep cycles. Thus, the light effects on the dynamics of SWA and REM sleep durations were blue shifted relative to the three-cone visual photopic system, probably mediated by the circadian, non-image-forming visual system. Our results can be interpreted in terms of an induction of a circadian phase delay and/or repercussions of a stronger alerting effect after blue light persisting into the sleep episode.

Introduction

The human circadian system is sensitive to non-visual effects of ocular light at short wavelengths via novel photoreceptors (Provencio *et al.*, 2000; Gooley *et al.*, 2001; Berson *et al.*, 2002; Hankins and Lucas, 2002; Hattar *et al.*, 2002). This so called 'non-image-forming' (NIF) system shows maximal response to light between 446 and 483 nm for melatonin suppression (Brainard *et al.*, 2001b; Thapan *et al.*, 2001; Wright and Lack, 2001), circadian phase shifting (Lockley *et al.*, 2003; Warman *et al.*, 2003), as well as reduction in cone b-wave implicit time of the human electroretinogram (Hankins and Lucas, 2002). Subjective alertness, heart rate and core body temperature increased significantly after blue (460 nm) but not after green (550 nm) light of equal photon density administered in the evening (Cajochen *et al.*, 2005).

In contrast to light's phase shifting effects (Czeisler *et al.*, 1989; Minors *et al.*, 1991; Khalsa *et al.*, 2003), less has been documented about the impact of white (polychromatic) light on human sleep architecture and spectral EEG density. Bright light in the morning shortened sleep duration (Dijk *et al.*, 1987b; Dijk *et al.*, 1989a) and advanced circadian rhythms without any effects on NREM sleep homeostasis (Dijk *et al.*, 1987b; Dijk *et al.*, 1989a; Carrier and Dumont, 1995). Wakefulness accumulated faster in the night following bright light treatment (Dijk *et al.*, 1987b) and sleep propensity was decreased during the first 90 min after evening light exposure, but only in the second post-exposure night (Carrier and Dumont, 1995). Bright light in the evening increased sleep latency to stage 2 (Drennan *et al.*, 1989; Cajochen *et al.*, 1992; Carrier and Dumont, 1995) and changed the time course of SWA, such that EEG delta power was lower during the first and higher during the fourth NREM-REM sleep cycle when compared to the dim light condition (Cajochen *et al.*, 1992). Others have reported shorter latency to REM sleep (RL) after bright light in the evening when compared to dim light conditions (Drennan *et al.*, 1989), or shorter RL after morning compared to evening bright light (Sack *et al.*, 1986). We hypothesized that changes in the sleep EEG depend on the wavelength of prior light administration. In order to test this hypothesis, we exposed volunteers to either monochromatic blue (460 nm) or green (550 nm) or to a dark condition (0 lux) in the evening, 2.5 hours before habitual bedtime.

Subjects and Methods

Study participants

Eight young male volunteers, (age range: 20-29 years; mean±SD: 24.6±3 years) completed the study. All were non-smokers, free from medical, psychiatric and sleep disorders, as assessed by a physical examination and questionnaires. To exclude visual impairments and ascertain that our light application was not harmful, an ophthalmological examination was carried out before and after the study (University Eye Clinic, Basel). For further details of the screening criteria see Cajochen *et al.* (Cajochen *et al.*, 2005).

One week prior to the study the volunteers were asked to abstain from excessive alcohol and caffeine consumption (i.e. at most 5 alcoholic beverages per week, and one cup of coffee or one caffeine-containing beverage per day). Furthermore, they were instructed to sleep approximately 8 hours per night and to keep a regular sleep-wake schedule (with bed- and wake times within ± 30min of self-selected target times between 10pm and 2am). The outpatient segment of the study was verified by a wrist actigraph (Cambridge Neurotechnologies®, Cambridge, UK), questionnaires and self-reported sleep logs. All volunteers confirmed their compliance by written informed consent. The protocol, questionnaires and consent form were approved by the Ethical Committee of Basel and conformed to the Declaration of Helsinki.

Study protocol and light exposure

The study consisted of three light conditions, which were carried out in weekly intervals in a balanced cross-over design with intra-subject comparisons. The volunteers were admitted to the laboratory 6.5 hours before their habitual bedtime (Figure 1). After preparation for polysomnographical sleep recordings, a constant posture protocol in bed in dim light (2 lux; polychromatic white light) was initiated. This 1.5-h episode of 2 lux was followed by 2 hours of dark adaptation (0 lux), and subsequently the subjects received monochromatic green (550 nm), blue (460 nm) or

no light (0 lux) for 2 hours. Following light exposure, the subjects remained awake for another 1.75 hour under 2 lux. Lights off was 1.25 hour after habitual bedtime, and the volunteers were allowed to sleep for 7.75 hours. The rationale for shifting bedtime 1.25 hours later was the requirement of monitoring the immediate after-effects of light exposure on physiological variables (melatonin, heart rate, CBT, alertness, etc.) before sleep onset, see (Cajochen *et al.*, 2005). During scheduled wakefulness, subjects were asked to answer numerous spoken questionnaires (Visual Analogue Scale, Karolinska Sleepiness Scale) and carry out the Psychomotor Vigilance Test. To further ensure wakefulness, a trained technician was constantly present in the adjacent room checking EEG traces on the screen display for sleep signs. Moreover, the subject's room was video controlled. Epochs of microsleep during scheduled wakefulness were also scored according to standard criteria (Rechtschaffen and Kales, 1968) and revealed neither a significant interaction between 'light condition' and 'time interval' nor significant main effects of those factors (p>0.1; two-way rANOVA: 'condition' x 'time interval', n=8; data not shown).

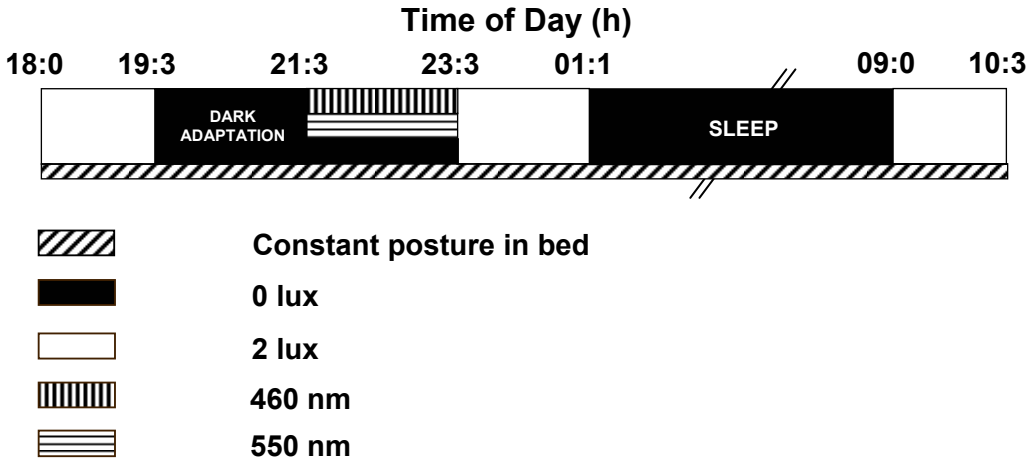


Figure 1

Overview of the protocol design. The different light conditions are dark (0 lux), dim light (2 lux), blue light (460 nm), and green light (550 nm). Time of day is indicated for a subject with habitual bedtimes between 24:00 and 8:00. The hatched bar designates constant posture in bed (e.g. recumbent during sleep and semi-recumbent during wakefulness).

The monochromatic light was generated by a 300-W arc-ozone-free Xenon lamp (Thermo Oriel, Spectra Physics, Stratford, CT, USA), filtered by either 460 nm or 550 nm with equal photon densities for both conditions (2.8 x 10^{13} photons/cm^2/s; interference filter, ±10 nm half-peak bandwidth, Spectra Physics Stratford, CT), the irradiance level was 12.1 µW/cm^2 for blue (460 nm) and 10.05 µW/cm^2 for green light (550 nm). The volunteers received the light via two glass fiber bundles (L.O.T. Oriel-Suisse, Romanel-sur-Morges, Switzerland) on custom-built goggles (K. Haug AG Basel, Switzerland). The study protocol and the light application have been described in detail elsewhere (Cajochen *et al.*, 2005).

Sleep Recording and Analysis

Sleep was recorded polysomnographically using the VITAPORT digital ambulatory system (Vitaport-3 digital recorder, TEMEC Instruments, B.V., Kerkrade, the Netherlands). Eight EEGs (F3, F4, C3, C4, P3, P4, O1, and O2), two electrooculograms, one submental electromyogram and one electrocardiogram were recorded. All signals were low-pass filtered at 30 Hz (4^{th} order Bessel anti-aliasing, total 24dB/Oct) at a time constant of 1 s before online digitization (range 610µV, 12bit AD converter, 0.15µV /bit) with a sampling rate of 128 Hz (for the EEG). The raw signals were stored online on a Flash RAM Card (Viking, USA) and offline downloaded to a PC hard drive.

Sleep stages were visually scored according to standard criteria (Rechtschaffen and Kales, 1968). All EEGs were subjected to spectral analysis using a FFT (Fast Fourier Transformation, 10% cosine 4s window) resulting in a 0.25-Hz bin resolution. EEG artifacts were automatically detected (CASA, 2000 PhyVision B. V., Gemert, NL). EEG power density was calculated during NREM sleep in the frequency range from 0 to 32 Hz. Here we report data derived from the right hemisphere (F4, C4, P4, O2) referenced against linked mastoids (A1, A2), in the frequency range between 0.5 and 20 Hz.

Thermometry

Core body temperature (CBT) was recorded continuously throughout the study, using a rectal probe with data collected every 20 s (Cajochen *et al.*, 2005). For statistical analysis, CBT was averaged over 20-min intervals per subject and condition.

Statistics

The statistical packages SAS® (SAS Institute Inc., Cary, NC, USA; Version 6.12) and Statistica® (StatSoft Inc., Tulsa, OK, USA; Version 6.1) were used. Visually scored sleep stages were expressed as percentage of total sleep time (TST) or in minutes (sleep latencies, total sleep time). For the accumulation curves, sleep stages were collapsed into 15 min intervals per condition for the first 6.75 hours.

Sleep EEG power density after the blue and green light exposure was expressed relative to EEG power density after the dark condition (log-ratios). Relative all-night sleep EEG spectra were analyzed during the longest common NREM sleep duration (1215 twenty second epochs ≅ 405 min) and re-transformed as percentage of the dark condition for graphical illustration. Furthermore, SWA was accumulated for the first 6.65 h of NREM sleep (=405 min), collapsed into 15-min bins and expressed as percentage of the accumulated value during 6.65 h after the dark condition (bottom panel of Figure 2).

NREM-REM sleep cycles were defined according to Feinberg and Floyd (Feinberg and Floyd, 1979), with the exception that for the last sleep cycle, no minimum REM sleep duration was required. Thereafter, each sleep cycle was subdivided into ten time intervals of equal length during NREM and into four time intervals during REM sleep and expressed as percentage of the dark condition. One-, two-, and three-way ANOVAs for repeated measures (rANOVA) with the factors ‘condition’ (blue, green, dark), ‘derivation’ (F4, C4, P4, O2), ‘cycle’ (1-3) and time intervals (15-min intervals) were used on log-transformed absolute values and on log-ratios. All *p*-values from rANOVAs were based on Huynh-Feldt’s corrected degrees of freedom and adjusted for multiple comparisons (Curran-Everett, 2000). For *post-hoc* comparisons, Duncan’s multiple range test with corrections for multiple

comparisons (Curran-Everett, 2000) was applied. All REM and NREM sleep durations per sleep cycle were analyzed with the Wilcoxon matched-pairs test, since not all of the values reached the criterion of a normal distribution.

To assess the decline of SWA (% of dark condition) across sleep cycles, a nonlinear regression analysis was calculated for each subject separately. SWA was approximated by an exponential decay function: $SWA_t=SWA_\infty+SWA_0*e^{(-r*t)}$, with SWA_t=averaged SWA per sleep cycle, SWA_∞=the horizontal asymptote for t=∞; SWA_0=the intercept on the y-axis and r=the slope of the decay and t=the time of each NREM cycle midpoint.

Results

Sleep stages

The visually scored sleep stages are summarized in Table 1. A one-way rANOVA with the factor 'condition' yielded significance for stage 4 ($F_{2,14}$=6.3; p<0.05) and slow wave sleep (SWS; $F_{2,14}$=7.9; p<0.05) and a tendency for stage 2 and 3 ($p\leq0.1$). *Post-hoc* comparisons revealed significantly less stage 4 and SWS after blue and significantly less SWS after green light when compared to the dark condition (p<0.05; Duncan's multiple range test). The accumulation curves showed a significant interaction between the factors 'condition' and 'interval' (1-27) for stage 3, 4, and SWS ($F_{52,364}$>2; p<0.05). Significant *post-hoc* comparisons between the blue and green light condition are indicated in Figure 2 (p<0.04 for SWS and p<0.01 for stage 3 and 4; Duncan's multiple range test and corrections for multiple comparisons). Hence, after the green light exposure, stage three was significantly decreased when compared to blue light. On the other hand, stage four was significantly lowered after the blue than after green light and SWS was altered during three time intervals across the night, with higher values during the first and lower values during the second and third of these intvervals, when compared to the blue light condition.

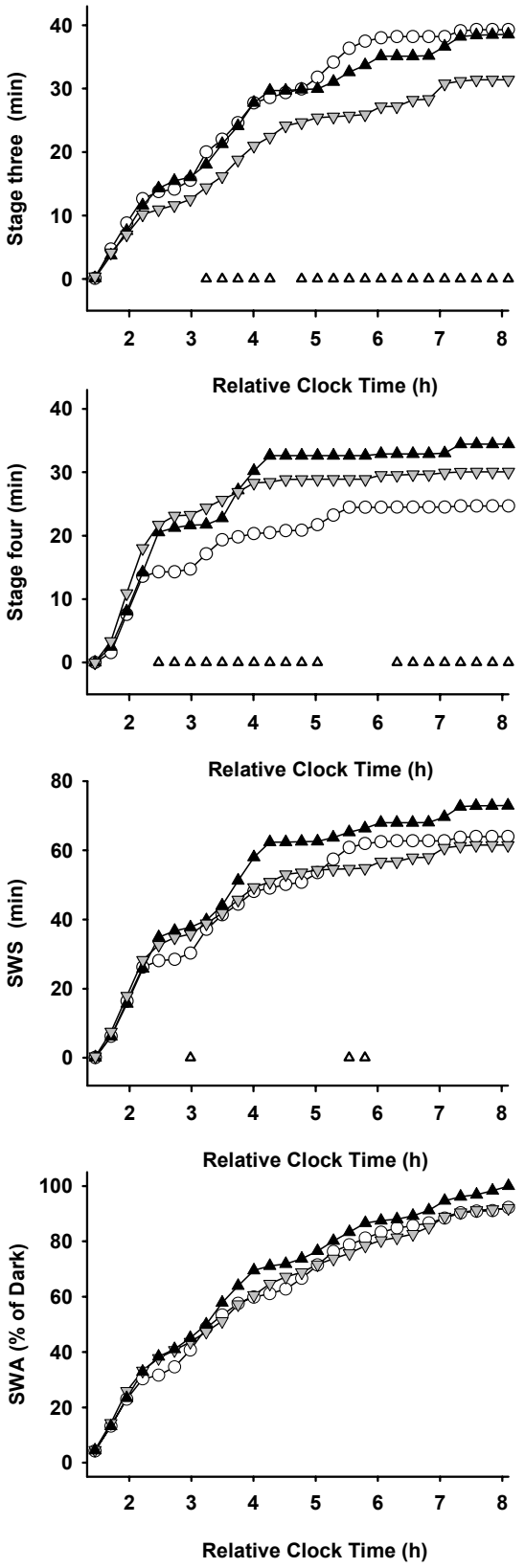
Table 1

Visually scored sleep stages. Values are means ± SE, n= 8.

Stage	Blue	Dark	Green	*P*	$F_{(2,14)}$
TST (min)	407.9±11.9	390.9±11.6	391.7±13.4	ns	-
Sleep efficiency (%)	87.8±2.5	84.1±2.5	84.3±2.9	ns	-
Stage One (%)	11.3±0.8	12.0±1.0	12.8±1.4	ns	-
Stage Two (%)	50.7±1.9	46.2±2.9	47.9±2.7	°	3.5
Stage Three (%)	10.0±1.0	10.0±0.8	8.0±0.7	°	3.3
Stage Four (%)	6.1±1.6	8.9±2.2	7.4±1.7	*	6.3
SWS (%)	16.1±2.2	18.9±2.8	15.5±2.2	*	7.9
REM (%)	21.8±1.6	22.9±1.5	23.9±1.7	ns	-
NREM (%)	66.8±1.4	65.1±1.2	63.4±1.3	ns	-
WALO (%)	12.0±2.4	14.8±2.4	14.9±2.9	ns	-
Arousal (%)	9.9±3.0	13.0±2.8	14.3±4.2	ns	-
MT (%)	1.1±0.6	1.9±0.8	1.7±0.4	ns	-
SL1 (min)	6.9±1.5	6.5±0.9	5.3±1.1	ns	-
SL2 (min)	8.8±1.4	9.0±1.1	7.7±1.1	ns	-
RL (min)	66.9±8.2	69.5±5.4	70.1±6.5	ns	-

TST, total sleep time (min); sleep efficiency [(stages 1-4 + REM)/(time after lights off - time lights on) x 100]; SWS, slow wave sleep (stages 3 + 4, % of TST); REM=rapid eye movement sleep (% of TST); NREM, non-rapid eye movement sleep [(SWS + stage 2)/TST] x 100; MT=movement time (after sleep onset; % of TST); WALO, wakefulness after lights off [(mt+wake)/(time after lights off - time lights on) x 100]; arousal, wake after sleep onset (% of TST); SL1, sleep latency to stage 1 (min); SL2, sleep latency to stage 2 (min); RL, REMS latency (after sleep onset; min); *=$p<0.05$, °=$p\leq 0.1$, ns=$p>0.1$ (one-way rANOVA).

Figure 2



Accumulation curves (collapsed into 15 min-intervals, for the first 6.75 hours) for stage 3, 4 and SWS. Accumulated SWA (% of dark condition, collapsed into 15 min-intervals) also are shown. Blue light (460 nm; open circles), green light (550 nm; grey triangles down) and dark conditions (0 lux; black triangles up) are indicated. Open triangles near the abscissa indicate intervals for which *post-hoc* comparisons between the blue and green light condition were significant (n=8; $p<0.05$; Duncan's multiple range test, corrected for multiple comparisons).

Sleep spectra

A two-way rANOVA on relative EEG power density values (log-ratios) with the factors ‘condition’ and ‘derivation’ revealed no significant interaction, nor did the main factor derivation yield significance (Figure 3). The main factor ‘condition’ yielded significance in the following frequency ranges: 3.25-3.5 Hz, 4.25-4.5 Hz, 6-6.25 Hz, 6.5-6.75 Hz, 7.75-8 Hz, 9-9.25 Hz, 11.5-12.5 Hz, 13.5-14 Hz, 14.25-15 Hz, 16.25-16.5 Hz (p<0.05; corrected for multiple comparisons). Hence, relative EEG power was significantly higher for the green than the blue light condition in these frequency ranges (one-way rANOVA; $F_{1,7}$ at least 5.6; p<0.05 on log-ratios; with the exception between 9-9.25 Hz where p<0.1). The accumulation of SWA in the course of the night (Figure 2, bottom panel) was calculated during the first 6.65 hours for C4 (see Methods section). There was no significant interaction with the factors time interval and condition, nor was the main factor condition significant (p>0.1; two-way rANOVA, on log-ratios).

Relative EEG power density per sleep cycle (% of respective dark cycle) for F4, C4, P4 and O2 are shown in Figure 4. Because all subjects completed three sleep cycles, the analysis was limited to these cycles. A significant three-way interaction between the factors ‘derivation’, ‘condition’ and ‘cycle’ occurred in the frequency ranges between: 1.25-2.75 Hz, 3-3.5 Hz, 3.75-4 Hz, 4.25-4.75 Hz, 7.75-8.25 Hz, 8.5-8.75 Hz, 11.75-12.25 Hz, 12.75-13.5 Hz, 15.75-16 Hz and 17-17.25 Hz (p<0.05; rANOVA on log-ratios; corrected for multiple comparisons; $F_{6,42}$ at least 2.6). The interaction of factors ‘condition’ x ‘cycle’ was significant in the frequency ranges between: 0.5-0.75 Hz, 1-3.5 Hz and 4-4.25 Hz (p<0.05; $F_{2,14}$ at least 4.3; corrected for multiple comparisons), and the main effect of condition was significant between: 9-9.25 Hz, 11.5-13 Hz, 13.5-15.25 Hz and 15.5-16.5 Hz (p<0.05; $F_{1,7}$ at least 6.1; adjusted to multiple measures). Post-hoc comparisons revealed significant differences between the third to both the first and the second sleep cycle for SWA, and the spindle and beta frequency range (see asterisks, Figure 4). These differences occurred mainly after blue light exposure in O2 (p<0.05, Duncan’s multiple range test, performed on log-ratios for each derivation and condition separately and corrected for multiple comparisons).

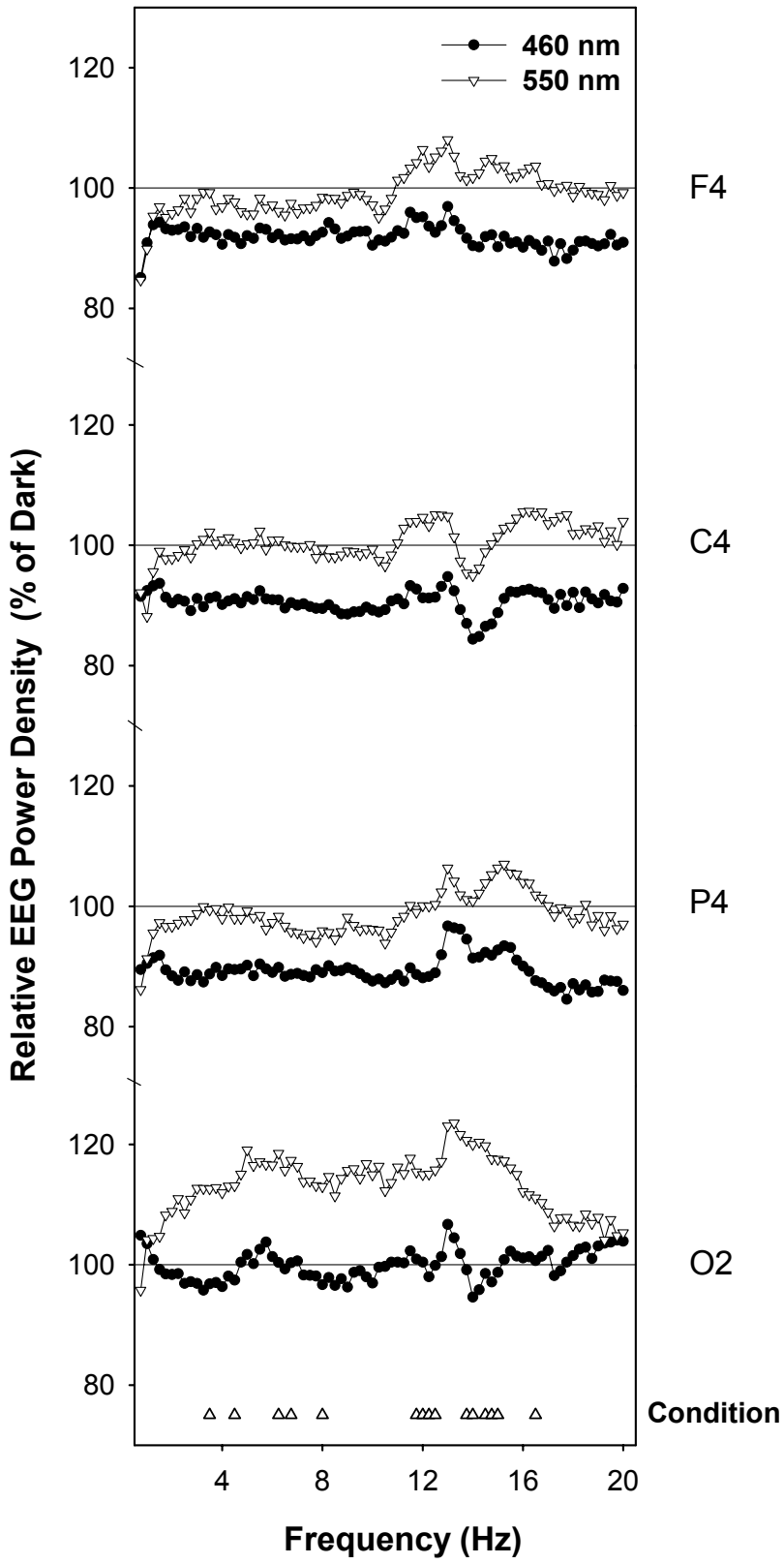


Figure 3

Relative EEG power densities (% of dark mean) during NREM sleep (405 min) in the frequency range between 0.5 and 20 Hz are shown for F4, C4, P4 and O2. Blue light (460 nm; black circles), and green light (550 nm; white triangles down) conditions are indicated (n=8). Open triangles near the abscissa indicate frequency bins for which EEG power density after green light was significantly higher than after blue light. $P<0.05$; one-way rANOVA; performed on log-ratios; p adjusted for multiple comparisons.

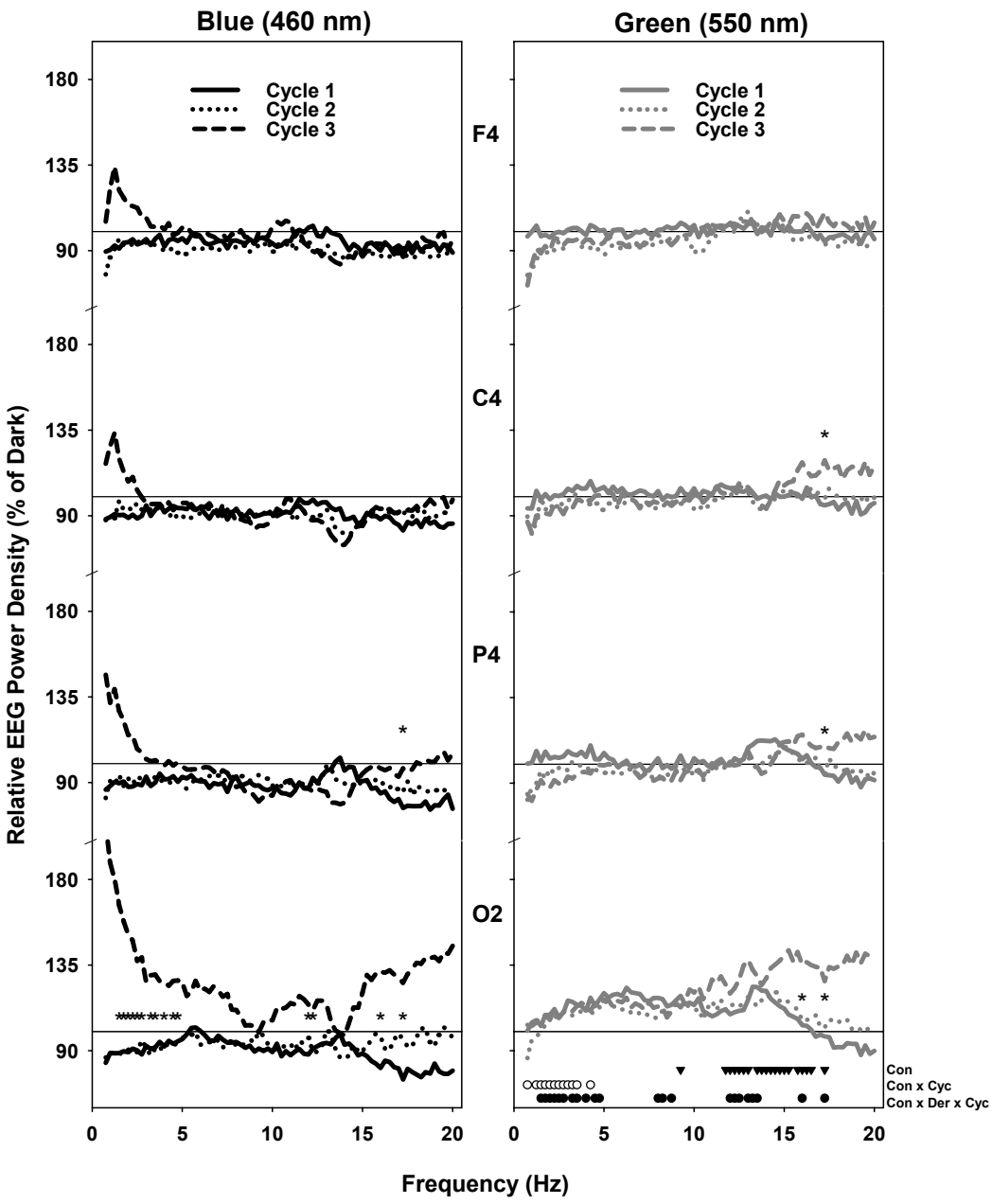


Figure 4

Relative EEG power density per NREM sleep cycle (% of respective dark condition) in the frequency range between 0.5 and 20 Hz. NREM sleep cycles 1-3 for blue light (460 nm; left) and green light exposure (550 nm; right) are shown for F4, C4, P4, O2. Filled circles at the bottom right indicate frequency bins, for which the 3-way interaction 'condition' x 'derivation' x 'cycle' (Con x Der x Cyc) was significant; open circles indicate significant interactions for 'condition' x 'cycle', and inverted triangles represent the significant main effect of 'condition' (p<0.05, corrected for multiple comparisons). *=p<0.05, cycle 1 vs. cycles 2 and 3 (p<0.05; Duncan's multiple range test, corrected for multiple measurements).

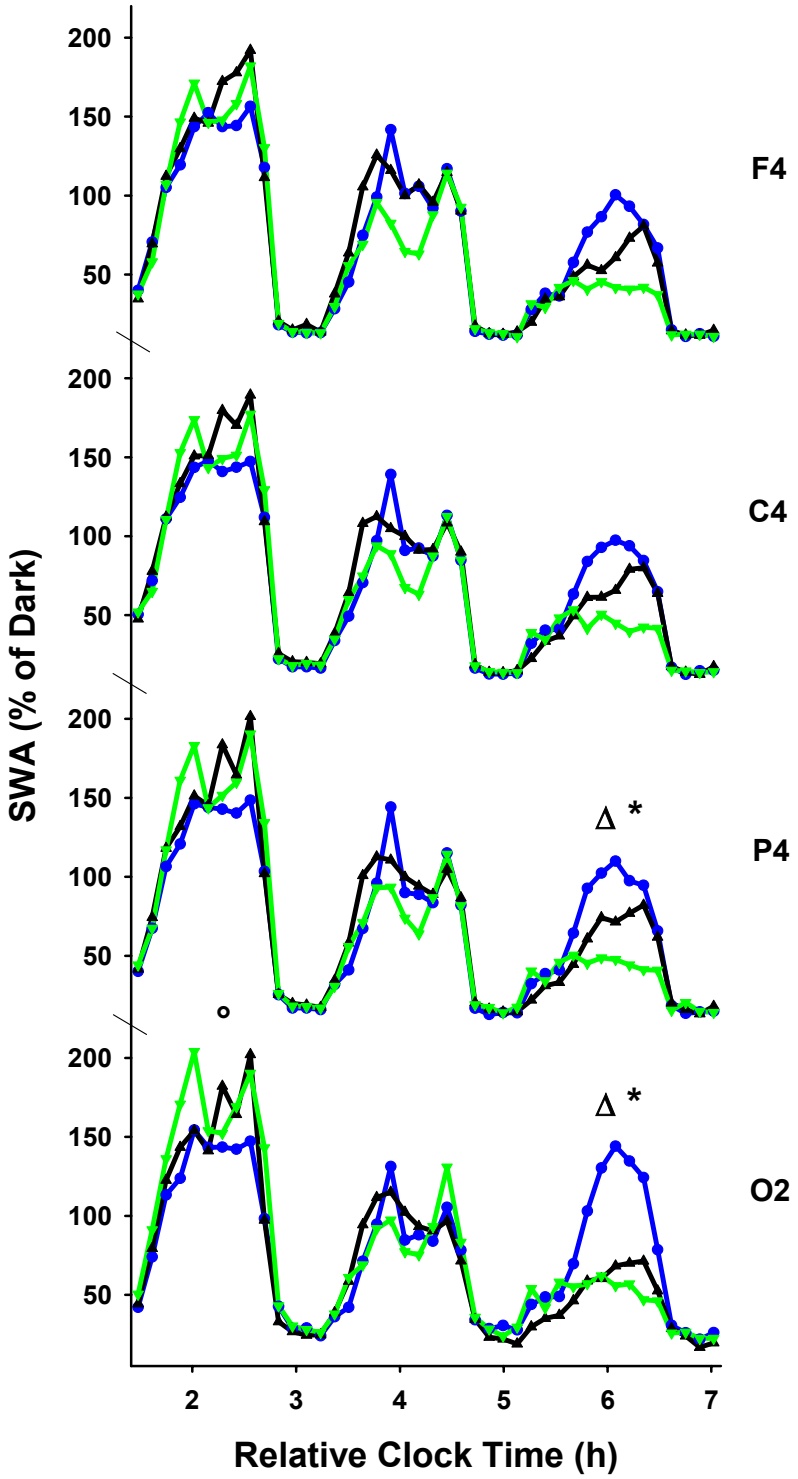


Figure 5

Dynamics of SWA per NREM-REM sleep cycle 1-3 for the derivations F4, C4, P4 and O2. Values are expressed as % of the dark condition and plotted against relative clock time (n=8) for blue light (460 nm, blue circles), green light (550 nm, green triangles down), and dark condition (0 lux, black triangles up). *= $p<0.05$; °= $p<0.1$ (green light vs. blue light), Δ= $p<0.05$ (blue light vs. dark condition).

To further analyze the time course of relative SWA across sleep cycles, SWA was expressed as percentage of the mean dark condition and plotted for each percentile (Figure 5). A two-way rANOVA ('condition' x 'cycle') performed for each derivation separately yielded significance in the parietal (P4; $p<0.05$; $F_{4,28}=3.6$) and in the occipital derivation (O2; $p<0.05$; $F_{4,28}=6.1$; performed on log-ratios). The main effect of 'cycle' was significant for F4, C4, P4 and O2 ($F_{2,14}>28$; $p<0.05$) whereas the factor 'condition' was not significant ($p>0.5$). *Post-hoc* analysis showed a tendency for less SWA after blue light during the first sleep cycle (in O2) when compared with the green (Duncan's multiple range test; $p=0.1$) and significantly higher SWA during the third sleep cycle after blue light in P4 and O2 when compared with the green and the dark condition (Duncan's multiple range test; $p<0.05$).

The fitted regression curves for mean SWA per NREM-REM sleep cycle are shown in Figure 6 for each derivation. The parameters of the decay functions revealed no significant interaction between the factors 'condition' x 'derivation' ($p>0.1$; two-way rANOVA). Only for the slopes the main factor 'derivation' yielded significance in O2 ($p<0.05$; $F_{3,63}=5.1$) when compared to F4, C4, and P4 ($p<0.05$; Duncan's multiple range test). T-tests performed between the three conditions (for each derivation separately) revealed no significance ($p>0.1$). From visual inspection, the slope after the blue light condition in O2 appears shallower than after the green or dark condition.

REM sleep duration (Table 2) was significantly shorter after blue light during the first sleep cycle when compared to the green light condition, and significantly shorter during the third sleep cycle in comparison to the dark condition ($p<0.05$) and tendend to be shorter ($p=0.06$) when compared to the green light condition (Wilcoxon matched-pairs test, on log-transformed data). There was no significant difference in REM sleep duration during the first sleep cycle between the green and the dark condition ($p>0.1$). On the other hand, NREM sleep duration was significantly shorter during the second sleep cycle after the blue light condition when compared to the green and dark conditions ($p<0.05$; Wilcoxon matched-pairs test).

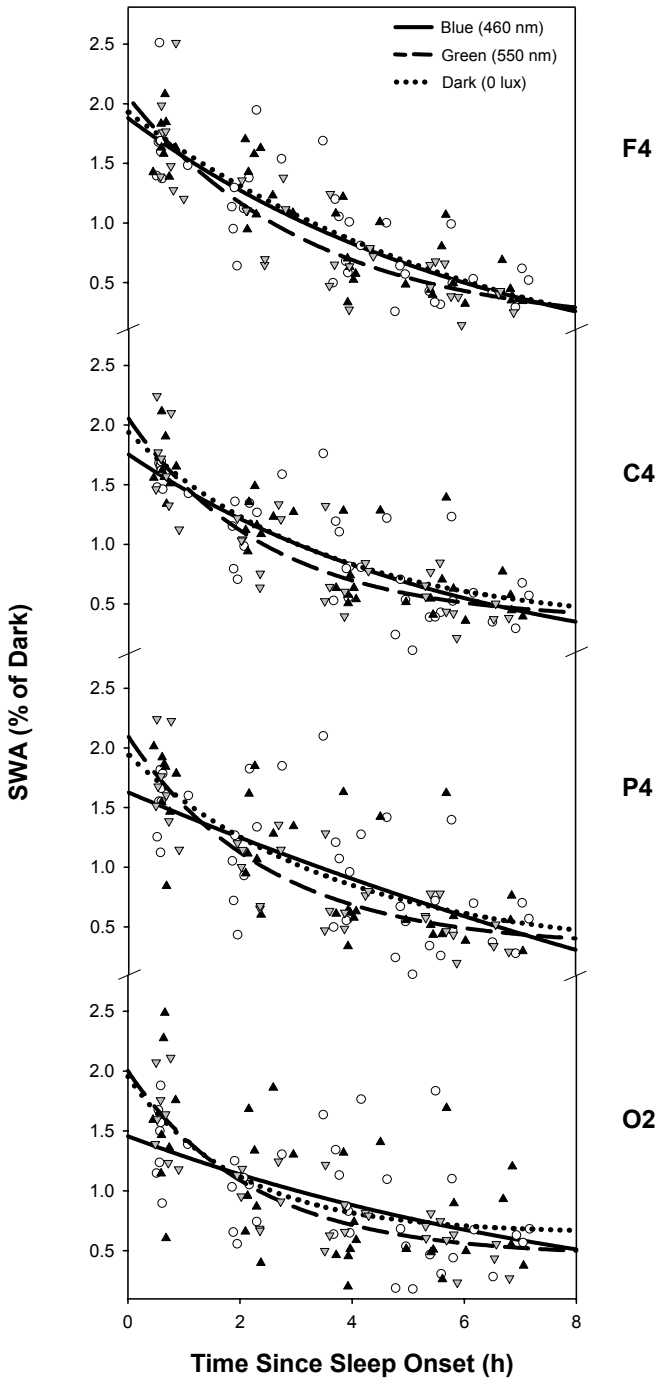


Figure 6

Time course of mean SWA in NREM sleep per NREM-REM sleep cycle along the antero-posterior axis (F4, C4, P4, O2). SWA was normalized to the dark condition. Solid, long dashed and dotted lines (resulting from the fitting function $SWA_t = SWA_\infty + SWA_0 \times e^{(-rt)}$, see methods for details) indicate the regression curves for blue light (460 nm), green light (550 nm), and dark conditions (0 lux), respectively. Data points representing blue light (open circles), green light (grey triangles down) and the dark condition (black triangles up) depict mean SWA per NREM-REM cycle for each subject.

Table 2

Mean REM sleep durations (min) per NREM-REM sleep cycles 1-3 for each light condition.

Condition	REM episode 1	REM episode 2	REM episode 3
Blue	13.5±2.3 [a]	22.9±15.2	12.9±4.1 [b,c]
Dark	17.3±4.5	22.1±2.6	35.1±9.7
Green	22.1±3.3	25.7.±7.4	33.9±9.9

Values are means±SE, n=8; a=p<0.05 for cycle 1, blue vs. green light condition; b=p<0.05 for cycle 3, blue vs. dark condition c=p<0.1 for cycle 3, blue vs. green light condition (Wilcoxon matched-pairs test).

Core Body Temperature

The time course of CBT for each condition after the dark adaptation is shown in Figure 7. The results of CBT before the sleep episode were reported in an earlier publication (Cajochen *et al.* 2005). After blue light, CBT tended to be higher for the first 40 min after lights off when compared to the green light condition (2-way rANOVA: 'condition' x 'time interval'; $F_{78,546}$=2.3; post-hoc comparisons: Duncan' multiple range test; p<0.1; adjusted for multiple comparisons). The next morning, CBT was significantly lower after the blue light compared with green light during the first 40 min after lights on and (p<0.05; statistics see above) and tended to stay lower for the next 40 min. Compared with the dark condition CBT tendend to be lower for the first 40 min after lights on after blue light (p<0.1), and was then significantly lower for the next 20 min (p<0.05) and tended to stay lower for the reminder of the study (p<0.1; for statistics see above).

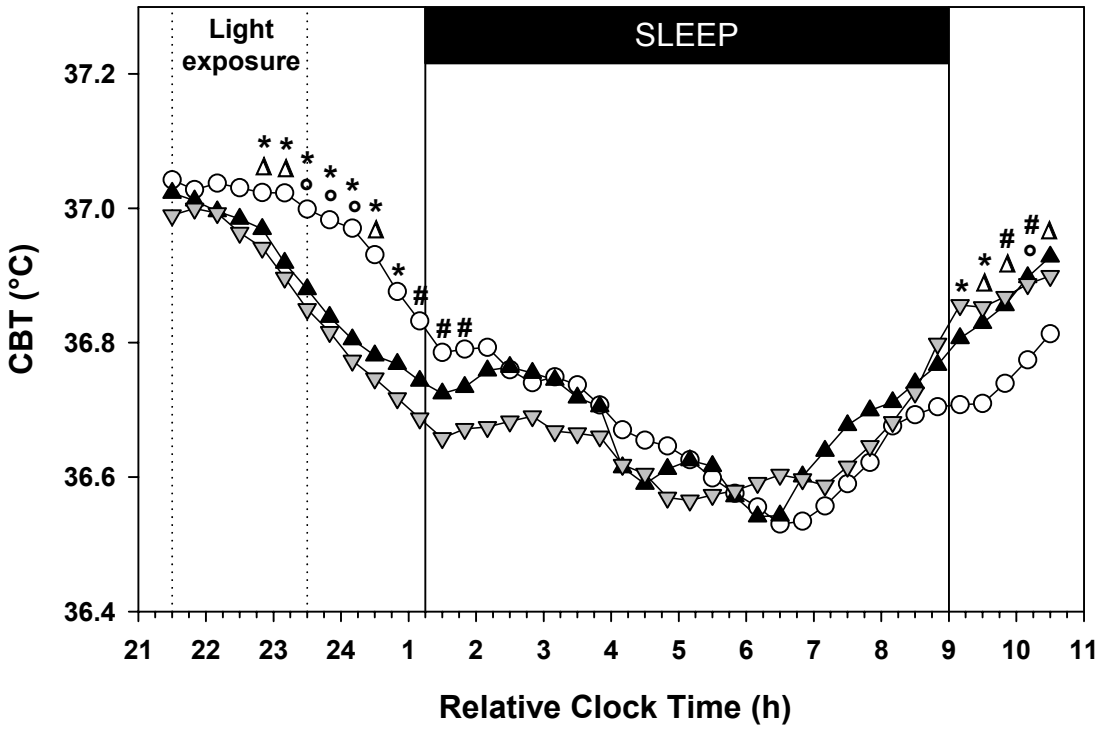


Figure 7

Time course of CBT (°C) during blue light (460 nm, open circles), green light (550 nm, grey triangles down) and dark condition (0 lux, black triangles up). °= $p<0.05$, Δ= $p<0.1$: blue light vs. dark condition. *=$p<0.05$, # =$p<0.1$: blue light vs. green light (n=8; for statistics, see text).

Discussion

Evening blue light exposure altered the time course of SWA across the post-exposure sleep episode, with somewhat lower values during the first and clearly higher values during the third NREM episode, particularly in parietal and occipital EEG derivations. REM sleep duration was significantly shorter after blue light exposure than after green light during the first sleep cycle and significantly shorter than in the dark condition in the third sleep cycle.

SWA during NREM sleep is maximal early in the sleep episode, declines exponentially as sleep progresses and is proportional to prior wake duration (Borbély, 1982). In our study the duration of prior wakefulness was identical for all three conditions, even though the 1.25-h delay in the timing of habitual lights off slightly enhanced homeostatic sleep pressure. Therefore, the observed changes in the time course of SWA after light exposure cannot be explained by alterations in prior wakefulness. The amount of EEG-recorded microsleeps during the light exposure did not significantly differ between conditions, and when accumulated over the entire sleep episode, SWA did not statistically differ between conditions. Thus, NREM sleep homeostasis does not seem to be dramatically altered by evening exposure to short-wavelength light. This is also corroborated by the fact that no significant interaction between ‘condition’ and ‘derivation’ for EEG power density during NREM sleep was found in any of the frequency bins between 0.5-20 Hz. However, EEG power density was significantly reduced in some frequency bins after blue light exposure when compared to the green light condition. This effect was rather unexpected, and could reflect a stronger decrease of EEG power density after blue than after the green light condition, independent of EEG derivation. The slightly decreased SWA during the first and significantly increased SWA during the third sleep cycle after the blue light condition may indicate either a continuation of the alerting effect found before lights off see (Cajochen *et al.*, 2005) with an intrasleep rebound of SWA during the third sleep cycle, or it may indicate the induction of a circadian phase delay. Support for the latter interpretation comes from the elevated CBT after blue light exposure, which lasted beyond the first 40 min following lights off. Additionally, the shortening of the first REM sleep duration (see below), as well as the markedly lowered CBT the next morning after lights on, could favor this argument that there was an immediate phase

delay after blue light exposure. However, our experiment was not designed for estimating circadian phase changes, and we did not measure any circadian phase markers 24 hours after light exposure on the following day.

REM sleep undergoes a marked circadian rhythm with high values in the second half of the biological night (Weitzman *et al.*, 1980). In our study, REM sleep duration did not differ between conditions across the entire night, but was shortened after blue light exposure during the first and third sleep cycle. The shortening of the first REM sleep cycle may again indicate a stronger phase delaying effect after blue than after green light. We found greater acute melatonin suppression concomitant with an increase in CBT during the evening exposure with blue light than with green light (Cajochen *et al.*, 2005). This effect lasted slightly beyond light exposure and lights off (see above). Exogenous melatonin administered in the late evening to healthy young subjects has been shown to prolong the duration of the first REM episode (Cajochen *et al.*, 1997; Cajochen *et al.*, 1998), along with a shorter latency to REM sleep (Cajochen *et al.*, 1997; Cajochen *et al.*, 1998). The same effect has been achieved in patients with reduced REM sleep, treated with melatonin (Kunz *et al.*, 2004). However, it remains to be elucidated whether there is a causal link between melatonin suppression, increased CBT immediately before and after sleep onset, and shorter REM sleep duration during the first sleep cycle after short wavelength light exposure. The shortening of the third REM sleep cycle after blue light could be related to the definition of sleep cycles (Feinberg and Floyd, 1979). According to Feinberg and Floyd (Feinberg and Floyd, 1979), a REM episode must contain at least 5 min of REM sleep. However, in two subjects the third REM episode was slightly shorter than 5 min after the blue light and counted as a REM episode, since there were no REM intrusions in the following NREM sleep episode.

The hypothesis that the NIF system is involved in these wavelength-dependent effects on sleep is supported by functional connections between the melanopsin-containing retinal ganglion cells of the NIF system to the circadian pacemaker in the suprachiasmatic nuclei (Gooley *et al.*, 2001; Berson *et al.*, 2002; Hannibal *et al.*, 2002; Hattar *et al.*, 2002), and to sleep-promoting neurons of the ventrolateral preoptic nucleus (VLPO) in the anterior hypothalamus (Gooley *et al.*, 2003). It has now been confirmed that melanopsin is the photopigment responsible for the non-image-forming system (Melyan *et al.*, 2005; Panda *et al.*, 2005; Qiu *et al.*,

2005). Based on the assumption that the NIF system is most sensitive to shorter wavelengths of light, its exposure would predict the strongest impact on brain areas, such as the VLPO, which contains sleep-promoting neurons. Hence, the inhibition of sleep-promoting neurons would be most pronounced after blue light. The (slightly) greater decrease of SWA during the first NREM cycle found after blue than after green and the dark condition could reflect such an impact of the NIF on sleep promoting brain areas.

It is known that polychromatic light has alerting effects in humans (Badia *et al.*, 1991; Cajochen *et al.*, 2000; Perrin *et al.*, 2004). Parallel analyses in the same experiment showed that 460 nm can induce such an alerting response already at these very low intensities (Cajochen *et al.*, 2005). This acute effect could have lasted beyond the beginning of the sleep episode, even though any such acute alerting and/or phase delaying effects after light exposure were not reflected in differences in sleep latencies, wake after sleep onset, sleep efficiency, and total sleep time. On the other hand, stage 3 and 4 were differentially affected by the two light conditions.

Differences in the time course of SWA were present only in more occipital derivations (P4, O2). If sleep is regarded not only as a global but also as a local brain phenomenon, the amount of SWA in a given brain region during sleep depends on how much it was ‘used’ while awake (i.e. SWA increases in those brain regions which were more strongly activated during prior wakefulness; Krueger and Obal Jr, 2003). Visual inputs of the image-forming system (rods, cones) reach the primary visual cortex in occipital brain regions via neuronal connections from the lateral geniculate nucleus of the thalamus (Hubel, 1988). Opsin-containing cells of the NIF system project to the lateral geniculate nucleus (Dacey *et al.*, 2005), and therefore information from both visual systems might be processed in the visual cortex. We could speculate that after blue light exposure, the ‘use’ of the primary visual cortex was greater than after green light or absolute darkness. However, since occipital SWA increased after blue light not at the beginning but later during the third sleep cycle, the concept of use-dependency does not appear appropriate here.

In conclusion, the wavelength-dependent effects of light on sleep architecture and EEG spectra were specific but rather small. They most likely resulted from an acute alerting effect continuing into sleep, and/or were a consequence of an immediate phase delay induced by blue light. Our results provide further evidence

that evening light exposure affects human physiology including sleep, and is dependent not only on duration and intensity but also on its wavelength via the NIF.

Acknowledgements

We thank Dr. Corina Schnitzler for medical and Prof. Selim Orgül for ophthalmological screenings, Claudia Renz, Marie-France Dattler and Giovanni Balestrieri for their assistance in data acquisition, and the subjects for participating. This research was supported by the Velux Foundation (Switzerland) and in part by Swiss National Foundation Science Grants START 3130-054991.98 and 3100-055385.98 to CC.

CHAPTER 6

General discussion

In this thesis, age-related changes in the circadian and homeostatic regulation of sleep, and the influence of monochromatic light on sleep architecture and EEG power spectra were studied. Regional differences of EEG power density between young and older subjects were found in the alpha, delta and spindle range, as well as differences in the time course of EEG delta activity. Older subjects felt sleepier in the afternoon and evening during the biological day (outside the phase of melatonin secretion). In parallel, they were able to sleep more during the wake maintenance zone than the young study participants, and exhibited decreased circadian melatonin secretion during the biological night.

The well known nocturnal decline of EEG SWA was changed after evening exposure to monochromatic blue light (460 nm), which was interpreted as the presumable induction of a phase delay and/or repercussions of the acute, alerting effect of prior light exposure.

Quantifying age-dependent homeostatic and circadian processes of sleep regulation

Homeostatic processes

Age-related differences in brain wave activity were assessed in two different protocols, where sleep pressure was either kept high (SD) or low (NP). Both protocols affected homeostatic and circadian processes differently with age. In response to elevated sleep pressure, the main finding was an attenuated frontal predominance of sleep EEG delta power. This could not be unambiguously interpreted as reflecting weaker homeostatic sleep regulation with age (see discussion in Chapter 2), because both age groups increased their EEG delta and theta power significantly in response to SD. One difference was a shallower decline

of EEG delta power throughout the night in older subjects, again predominantly in frontal brain areas. Within this context, the possible age-related lowering of the cortical arousal threshold during sleep in mainly frontal brain areas was discussed. Evidence for this attenuation in older persons has been attained in studies of the arousal threshold in response to auditory stimuli during sleep (Zepelin *et al.*, 1980; Mc Donald *et al.*, 1981). In a forced desynchrony study, older participants had higher wakefulness within scheduled sleep episodes at all circadian phases (Dijk *et al.*, 1999a). An assumed age-related dampening of the cortical arousal threshold could interfere with local, use- or experience-dependent processes during recovery sleep. As differences in the sleep EEG in mainly frontal brain areas emerged only under SD conditions, they may reflect the higher age-dependent vulnerability to SD (‘prefrontal tiredness’).

A possible argument for weakened homeostatic sleep regulatory processes with age are the subjective sleepiness levels during both CR and NP protocols. The elderly participants were more alert in the morning but sleepier in the evening of the first and second day than the young (Figure 1). One argument might be that under ‘normal’ sleep pressure conditions the elderly exhibit a faster homeostatic increase of sleep pressure during the wake episode. However, it is interesting to see no significant differences in subjective sleepiness between young and older subjects when wakefulness lasted beyond the usual 16 hours (i.e. during SD; Figure 1). In line with these results are the performance data from the Psychomoter Vigilance Test (PVT). Here, the 10% slowest reaction times are impaired during the first 16 hours but not after longer wakefulness (Blatter *et al.*, 2006). Hence, older subjects do not appear to be more impaired after prolonged wakefulness than the young. This has also been reported in young and older men (Adam *et al.*, 2006), where the elderly attained even better performance than the young after more than 16 hours of wakefulness. Age-related changes in subjective alertness and PVT performance might be due to an altered, motivational behaviour with age, as subjective alertness is highly correlated with motivation, at least in young subjects (Hull *et al.*, 2003).

Changes in the dynamics of the sleep EEG under low sleep pressure, illustrated by the shallower decline of EEG delta in the older group already present during the BL (Chapter 2), also corroborates results from other studies (Landolt *et al.*,

1996; Dijk *et al.*, 1999b). When homeostatic sleep pressure was kept low, both age groups lowered their EEG delta power equally during the first recovery NREM cycle,

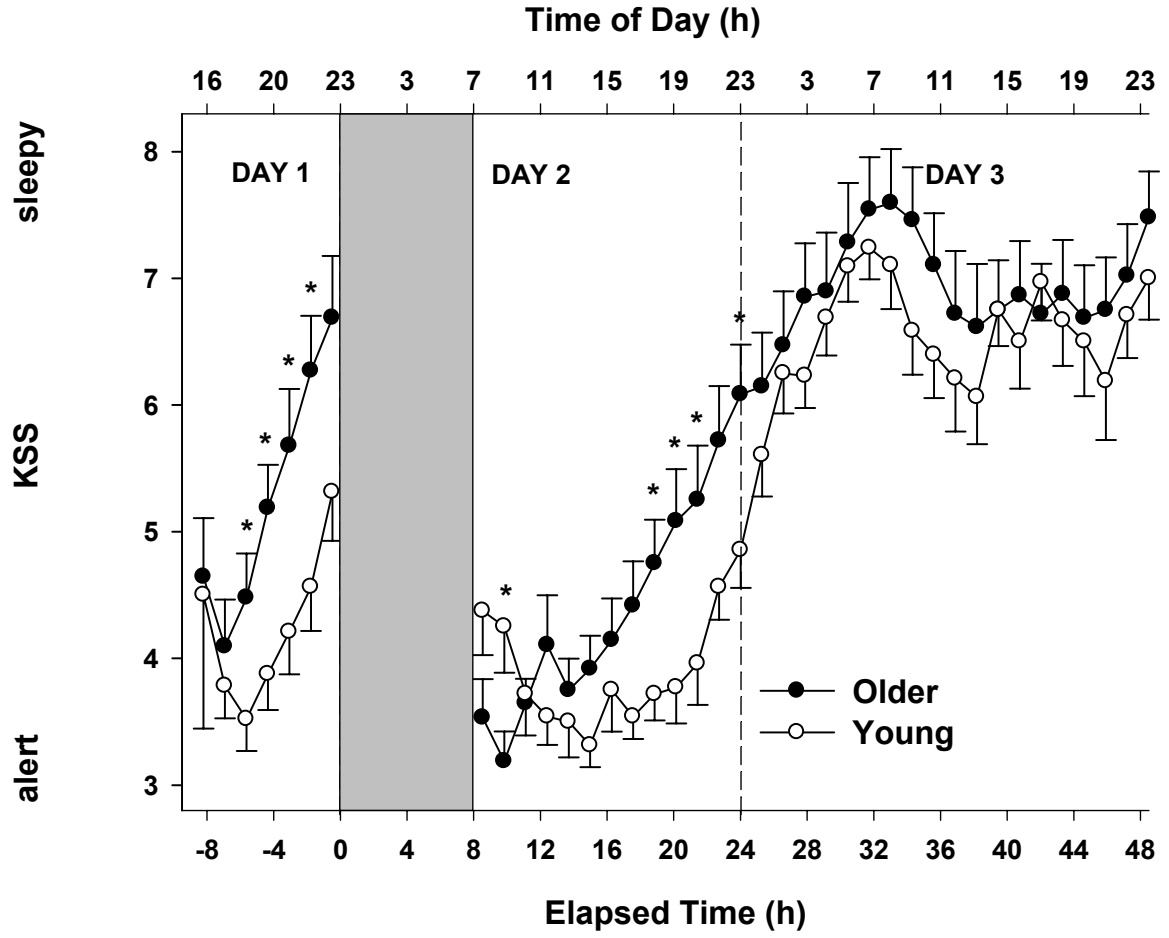


Figure 1

Subjective sleepiness levels (assessed by the Karolinska Sleepiness Scale, KSS) are indicated for the young (open circles; n=16; mean-SEM) and the older (filled circles, n=16; mean+SEM) in 1.25-h intervals. The values are shown for the first 8 hours before the BL night, and during the 40 hours of sustained wakefulness after the BL night, all under CR conditions (see Chapter 2). Asterisks depict significant differences between young and older subjects (Mann-Whitney U test; $p<0.05$, corrected for multiple comparisons after Curran-Everitt, 2000).

as predicted by the model (Borbély, 1982; Daan *et al.*, 1984; Dijk *et al.*, 1987a). However, this response was significantly shorter-lived in the elderly (only the first NREM episode) than in the young participants (across the first two NREM episodes), and we argued (Chapter 4) that this could not unambiguously be attributed to a weaker homeostatic sleep regulation, because the initial level of relative EEG delta activity (e.g. during the first NREM cycle) was similar in both age groups. Similarly, Campbell and Feinberg found no differences in the EEG delta decline between young and older subjects during a post-nap night (Campbell and Feinberg, 2005). Whether changes in the dynamics of the EEG delta decline indicate a shorter-lasting satiation of homeostatic components with a subsequent intra-sleep rebound, is still not clear. One study reported an age-related prolonged response to SD of stage 4 increase, Taken together, it appears that homeostatic sleep regulation is still operational with age – even though at a lower level (SWA decrease) and with an altered time course. Hence, one key element for future studies with older persons could be the more detailed analysis of short and long lasting age-related homeostatic effects (e.g. dose response curves), objective measures during wakefulness (the wake EEG) and the combination with functional molecular variables (e.g. adenosine expression).

Circadian processes

Several age-related circadian facets emerged under nap conditions, where homeostatic sleep pressure was kept low. The circadian rhythm of melatonin secretion was lower in the elderly, and the temperature amplitude went in the same direction (unpublished data). Our older participants did not show a significant circadian phase advance, when compared to young, neither were their phase angles changed (Chapter 3), as has been previously found (Duffy *et al.*, 1998; Duffy *et al.*, 2002). A possible explanation for the non-advanced circadian phase are the screening criteria, where extreme morning types had been excluded. Other reasons might be the lower age range of our older subjects when compared to the age range in studies which did find differences in the phase angles (Duffy *et al.*, 1998; Duffy *et al.*, 2002), or the earlier habitual bed- and wake times in our young cohort, when compared to theirs (11:34pm ±56 min vs. 12:14pm±53 min; mean±SD).

The EEG spindle and lower alpha range exhibited less pronounced night-day differences (i.e. in-, and outside the window of melatonin secretion) in the older than in the young group, with an attenuated nocturnal peak in the lower spindle frequency range and less nocturnal reduction in the high spindle frequency range (Chapter 3). Quantitative sleep EEG analyses of young and older subjects by means of fast time frequency transform (FTFT), which allows the detailed segregation of different spindle variables, revealed significantly higher spindle frequencies during the day only in the younger group (Knoblauch *et al.*, 2005). Moreover, the circadian variation of sleep spindles and sleep propensity were more weakly coupled with the circadian variation of melatonin secretion in the elderly, such that spindle frequency appeared advanced with respect to the melatonin rhythm (Knoblauch *et al.*, 2005, Figure 2). Therefore, it was hypothesized that the circadian modulation of spindle frequency could facilitate the modulation of arousal and sleep propensity at the appropriate times of day in the young, but less so in older subjects (Cajochen *et al.*, 2006b). Evidence for lower circadian amplitude emerged from the multiple nap study, where under low sleep pressure the elderly revealed a diminished circadian rhythm in REM sleep, sleep propensity (Chapter 3), and an earlier spindle frequency reduction in the evening (during the wake maintenance zone; Knoblauch *et al.*, 2005). In parallel, subjective sleepiness levels were higher in the elderly and they were able to sleep at this time of day, whereas young subjects rarely did so (Chapter 3), also corroborating findings with ultra-short (7/13 min) sleep-wake cycles across 24h (Lavie, 1986). The often reported age-related difficulties in maintaining sleep during early morning hours, when sleep pressure dissipates, was not reflected in sleep propensity of our elderly volunteers, nor did we find a clear correlation between sleep propensity and spindle frequency in the older group (Knoblauch *et al.*, 2005). However, visual inspection of Figure 7 suggests an earlier increase of spindle frequency in the older than in the young, relative to melatonin secretion, which may indicate facilitated awakenings from sleep with age. The latter has also been reported in FD studies with young and older subjects, where the older cohort had most awakenings when the last sleep episode coincided with the end of the nocturnal melatonin peak (Dijk *et al.*, 1999a).

Our results revealed diminished circadian amplitude of various parameters with age, but we have obviously no proof of whether it is the clock itself (i.e. the

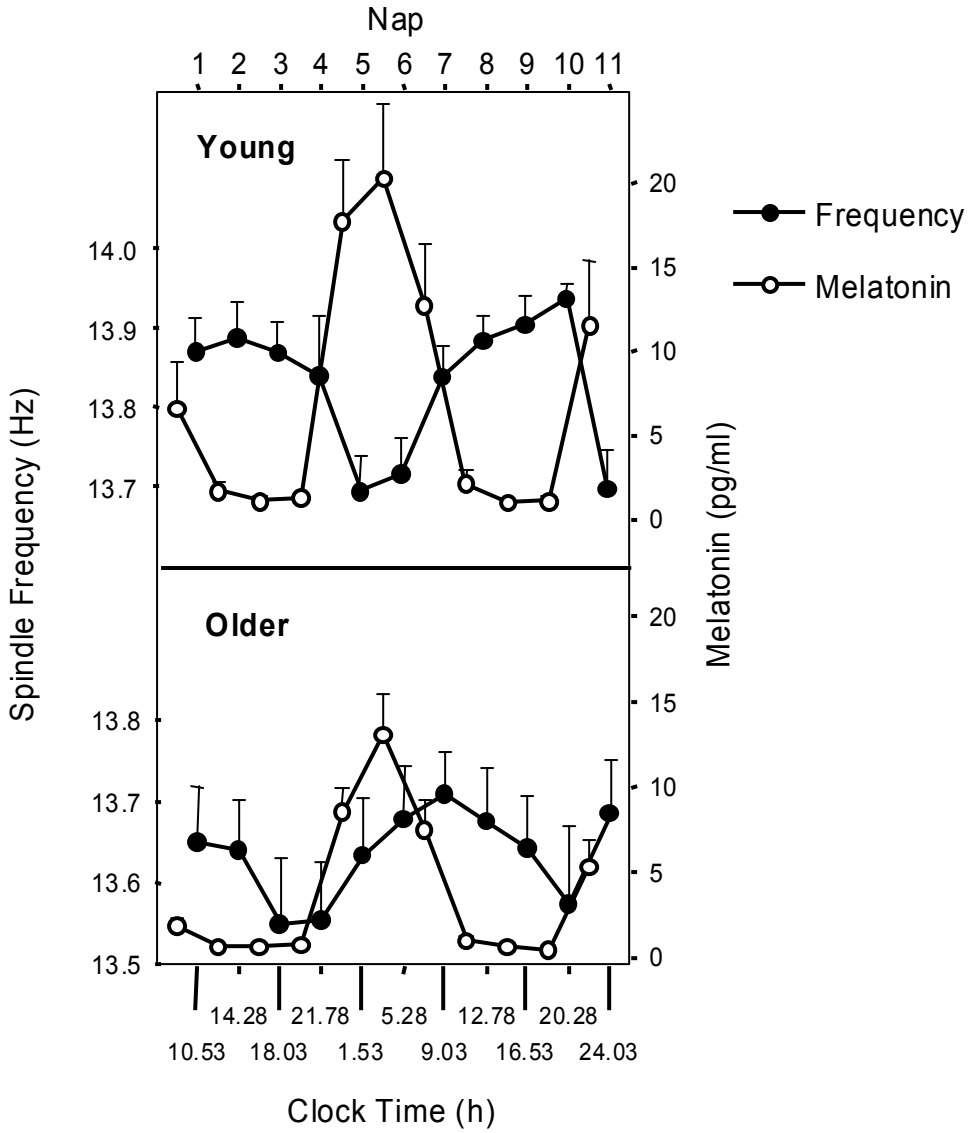


Figure 2

Dynamics of the spindle frequency (filled circles) and salivary melatonin levels (open circles) are shown for young (upper panel) and older participants (lower panel; mean + or - SEM). The averaged mean per nap comprised at least 10 subjects for the older and 12 for the young group, except for nap 4 (n=5) and nap 10 (n=3) within the young subjects (with permission from Knoblauch *et. al.* 2005).

responsiveness of the SCN) which decreases with age, or whether afferent or efferent signalling pathways of the SCN are impaired (Mistlberger, 2005). If afferent pathways from to the SCN undergo an age-related impairment, then also the zeitgeber strength e.g. of light, would be affected. This is known to be a consequence of visual impairments (see Chapter 1), since retinal light transmission, especially in the shorter wavelength ranges (see Chapter 5), declines with age. The reduced physiological response to shorter wavelengths of light in healthy elderly was shown after blue-light exposure in the evening, where healthy older participants exhibited less melatonin suppression when compared to young controls (Herljevic *et al.*, 2005).

In favour of clock-related impairments is the evidence from post-mortem tissue of human SCNs which have revealed general, age-related cell degeneration (Hofman and Swaab, 2005).

The 'downstream' signalling pathway from the SCN to sleep- and wake promoting brain areas (see also Chapter 1) may also be affected. The state stability of sleep and wakefulness in the proposed flip-flop system (Saper *et al.*, 2001) could be impaired with age in several ways. As orexin-containing neurons serve to stabilise wakefulness (Saper *et al.*, 2001) they are also under the control of the SCN (Abrahamson *et al.*, 2001) and their expression shows diurnal variation with highest values during the active phase, peaking in the wake maintenance zone in primates; (Zeitzer *et al.*, 2003) and lowest during rest episodes in rats and primates (Yoshida *et al.*, 2001; Zeitzer *et al.*, 2003). There is growing evidence from other animal studies that orexin A- and B-producing neurons of the lateral and posterior hypothalamus are not only diminished in number but also in the respective gene expression levels in aged rat brains (Porkka-Heiskanen *et al.*, 2004). The authors emphasize the characteristics of ageing: decreased age-related consolidation of vigilance states, endocrine changes and dysfunctions of the autonomous nervous system (Porkka-Heiskanen *et al.*, 2004).

Taken together, ageing humans undergo impairments of the circadian timing system, such that beside several physiological and behavioural functions, sleep timing, consolidation and structure are also affected. These deteriorations become most clearly manifested when the adjustment of the circadian system is most needed, i.e. in the late evening, when homeostatic sleep pressure is high and in the early morning, when homoeostatic pressure has dissipated (Figure 3). We favour the

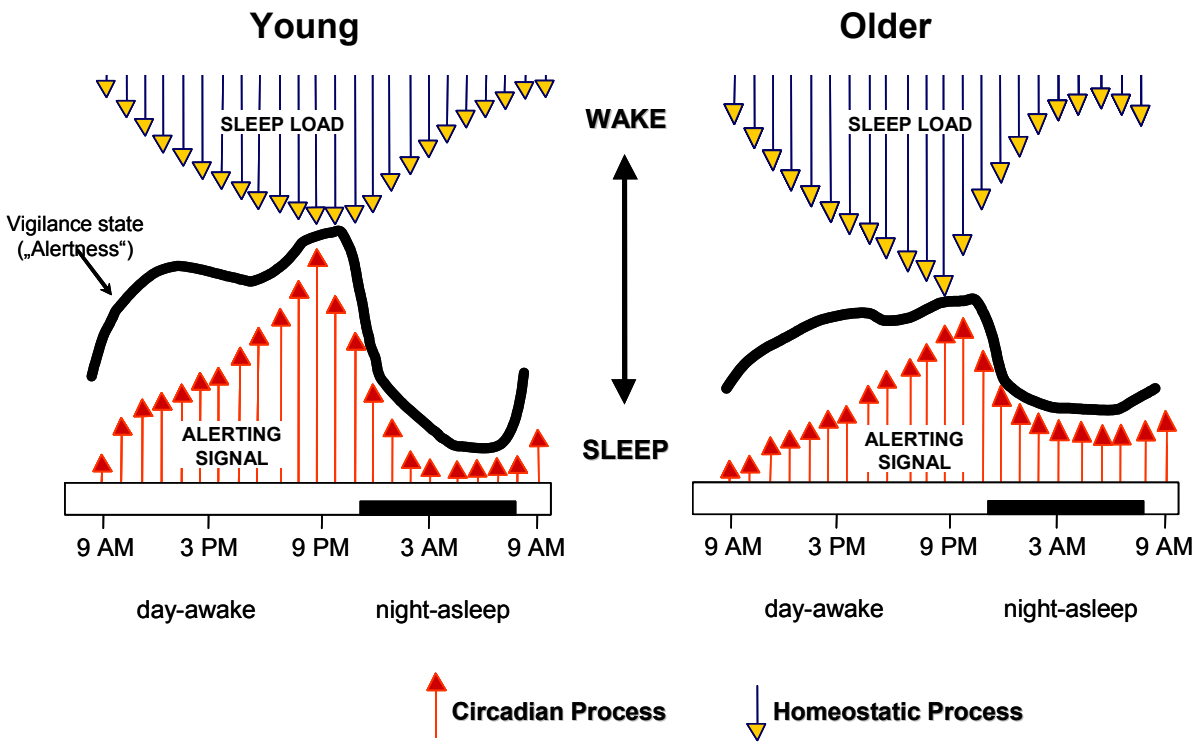


Figure 3

Visualization of the age-related attenuation of circadian and/or homeostatic processes (‘sleep load’) in humans, assessed by the subjective vigilance state for young (left hand panel) and older subjects (right hand panel). Arrows upwards indicate the circadian alerting signal, peaking in the late evening with a nadir in the early morning hours. Please note that the circadian alerting signal never entirely disappears. Arrows downward represent the sleep load, which shows the same extremes as the circadian signal but in the opposite direction (adapted from Edgar, 1993).

idea that the circadian system is weakened and thus the appropriately opposing mechanisms cannot sufficiently balance the homeostatic process, leading to age-related decrements in sleep-wake behaviour.

Influence of monochromatic light exposure on sleep

Evening exposure of monochromatic light changed the time course of EEG SWA with a decrease during the first and an increase during the third NREM/REM sleep cycle (Chapter 5). This was interpreted as a stronger acute and/or circadian phase delaying effect after blue light exposure. The effects were similar to those found more than ten years earlier with polychromatic bright light of much higher intensity (Cajochen *et al.*, 1992). Even though we did not dilate pupils artificially (as was done in the landmark experiment, Brainard *et al.*, 2001b), we found in a control experiment that blue light exposure actually resulted in more constricted pupils (Cajochen *et al.*, 2005), in spite of having stronger effects on sleep (Chapter 5), alertness, CBT, heart rate and melatonin than after the green light condition (Cajochen *et al.*, 2005). Thus, despite the presumably lower input of photons during blue light exposure, the elicited responses were stronger and longer-lasting than after green or no light exposure. These effects on pupil reaction have been attributed to the melanopsin-containing RGCs in animal studies (Lucas *et al.*, 2001), but have so far not been reported for humans. In the same experiment, we also collected mucosa samples in a non-invasive procedure for clock gene analyses. Compared to the normal 24-h time course (under dim light conditions), we could demonstrate that blue light stimulated PER2 gene expression significantly more strongly than green light at a time when melatonin was most suppressed by blue light (Cajochen *et al.*, 2006a).

We do not consider that other than circadian effects on sleep occurred (see Discussion in Chapter 5), even though several EEG parameters were differently affected by light. Some frequency bins of the all night EEG spectra were significantly lower after the blue when compared to the green light condition (Chapter 5), an effect which was unexpected, and not previously reported in the literature. On the other hand, visual scoring revealed a decrease of SWS after both light conditions, which came about via the significant reduction of stage 3 after green and the reduced stage

4 after blue light, indicating a gradient of visually scored delta waves from green to blue light. Whether such 'secondary' results hint towards additional functions of the NIF on human sleep, remains to be elucidated. Interestingly, in nocturnal animals (mice), monochromatic light at night elicited an increase of SWS under controlled experimental conditions, and thus had sleep promoting effects (R. Foster, personal communication).

Perspectives

Sleep is important for health and quality of life at all ages. The outcome of our study with healthy older volunteers suggests that manipulations of both circadian and homeostatic processes could improve age-related decrements in sleep and daytime alertness levels. A potential strategy to manipulate the circadian timing system is given by increasing zeitgeber strength, such that longer and higher (daytime) light intensities could, when given at the right time, ameliorate nocturnal sleep and daytime alertness levels, without side effects (for a review see Van Someren, 2000). This has already been shown with aged demented patients (Ancoli-Israel *et al.*, 2003). In patients suffering from seasonal affective disorder light has an antidepressive effect (Wirz-Justice *et al.*, 2005). The efficacy of bright light therapy may be improved by using light enriched in the shorter spectral wavelength range as it has been shown by Brainard and coworkers (Glickman *et al.*), or by filtering these wavelengths from normal polychromatic daylight by means of special lens systems. The wavelength of light seems to be crucial. In a recent study of older depressed patients no statistical difference between light treatment with green (500 nm) and placebo condition (red light) was found (Loving *et al.*, 2005). Further applications of blue-enriched light could be, similar to the known applications of polychromatic white light, to alleviate circadian disentrainment after shift work, jet lag, or light therapy in non-seasonal depressed patients. Before such applications are ready for commercial use, carefully conducted studies must supply evidence of (long-term) safety (e.g. the well known blue-light hazard) and concomitantly, the knowledge of other physiological functions of the circadian timing system should be extended.

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CURRICULUM VITAE

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- Cerebral rhythms (2003)
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- Neuropsychology of memory disorders and dementia (2004)
- Cognitive Neurosciences: methods and applications (2004)
- Chronobiology in sleep and medicine (2004)
- Cortical motor control (2005)
- Cerebral processing of emotions (2005)
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Others

1984-1987 Diploma in General Nursing (AKP), Chur (Switzerland)

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Münch M, Knoblauch V, Blatter, K, Kräuchi K, Wirz-Justice A, Cajochen C. Homeostatic sleep regulation under low sleep pressure: are there age effects? Swiss Society for Neuroscience (SSN), Basel (Switzerland), January 2006.

Oral and poster presentations at national and international meetings

2003 Is the circadian arousal signal in the evening attenuated in aging? Poster presenter at the Gordon Conference for Chronobiology, Barga (Italy), May.

2003 Age related attenuation of the circadian arousal signal in the late evening. Poster presenter at the Joint Annual Meeting Swiss Society of Sleep Research, Sleep Medicine and Chronobiology & Swiss Society for Neurology, Zürich, (Switzerland), November.

2004 Age-related attenuation of the circadian arousal signal in the late evening. Joint Meeting. Poster presenter at the Swiss Society for Neuroscience and Swiss Society of Psychiatry and Psychotherapy Meeting, Lausanne (Switzerland), January.

2004 Age attenuates the daytime circadian arousal signal. Poster presentater at the Basel Neuroscience Symposium: 'From bench to bedside', Basel (Switzerland), September.

2004 The frontal predominance of EEG delta response to sleep loss decreases with age. Oral presentation at the 17th Meeting of the European Sleep Research Society (ESRS), Prague (Czech Republic), October.

2004 The frontal predominance of EEG delta response to sleep loss decreases with age. Poster presenter at the Swiss Society for Sleep Research, Sleep Medicine and Chronobiology Meeting, Schlafschiff Basel (Switzerland), December.

2005 The frontal predominance of EEG delta response to sleep loss decreases with age. Poster presenter at the annual meeting of the Society for Neuroscience (SSN), Zürich (Switzerland), February.

2005 Wavelength-dependent light effects on sleep architecture and sleep EEG power density in humans. Poster presenter at the Swiss Society for Sleep Research, Sleep Medicine and Chronobiology (SGSSC) Annual Meeting, Lucerne (Switzerland), April.

2005 Wavelength-dependent light effects on sleep architecture and sleep EEG power density in humans. Oral presentation at the Associated Professional Sleep Societies (APSS) 19th Annual Meeting, Denver CO (USA), June.

2005 Wavelength-dependent effects of evening light on sleep architecture and EEG slow-wave activity. Poster presenter at the Society for Light Treatment and Biological Rhythms (SLTBR) Annual Meeting, Eindhoven (Netherland), July.

2005 Wavelength-dependent effects of evening light on sleep architecture and EEG slow-wave activity. Poster presenter at the European Pineal and Biological Rhythms Society (EPBRS) Meeting, Frankfurt (Germany), September.

2006 Homeostatic sleep regulation under low sleep pressure: are there age effects? Poster presenter at the Swiss Society for Neuroscience (SSN), Basel (Switzerland), January.

2006 Age-related changes in homeostatic sleep regulation after multiple naps. Poster presenter at the Neurex Annual Meeting, Basel (Switzerland), March.

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Awards and Travel Grant

Poster prize at the Swiss Society for Sleep Research, Sleep Medicine and Chronobiology (SGSSC) and the Swiss Society for Neurology (SGN) Meeting in Zürich (Switzerland), November 2003.

Helgi Kristbjanarson award for the oral presentation at the 17. Congress of the European Sleep Research Society, Prague (Czech Republic), October 2004.

Travel grant from the University of Basel (Switzerland) for the visit to Brigham and Women's Hospital, Harvard Medical School, Boston, MA (USA), January 2006.

Memberships in Professional Societies

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since 2006:	Student member of the Society for Sleep Research (SRS)
since 2006:	Student member of the Swiss Society for Sleep Medicine and Chronobiology (SGSSC)

ACKNOWLEDGEMENTS

The present thesis was realized at the Centre for Chronobiology of the Psychiatric University Clinics of Basel, under the supervision of Prof. Dr. Christian Cajochen and Prof. Dr. Anna Wirz-Justice.

First I would like to warmly thank Prof. Dr. Anna Wirz-Justice as head of the lab for her powerful support throughout the entire time of my thesis, her suggestions and fruitful discussions, as well as her passionate interest in all applications of chronobiological research. I am deeply grateful to Prof. Dr. Christian Cajochen, who was the principal investigator of the projects and the first supervisor of my thesis, for his excellent support. He thoroughly taught, instructed and accompanied me through all steps of the work, from project planning up to data analysis and writing scientific articles. I owe both warm thanks for their generosity that enabled me to actively participate in numerous scientific congresses and meetings.

I very much thank Dr. Vera Knoblauch for her great introduction to data evaluation and analysis and her advice in practical questions. To Kurt Kräuchi I am grateful for his assistance in statistical questions and for the numerous discussions and suggestions. To all other former and present colleagues I would like express thanks for a positive, helpful working atmosphere as well as stimulating discussions. I very much appreciate the invaluable support of our technicians Claudia Renz, Marie-France Dattler and Giovanni Balestrieri, for without their help the studies could not have been run. I am also grateful to the Psychiatric University Clinics Basel (Director: lic. jur. Fritz Jenni, Medical Director: Dr. Franz Müller-Spahn) for generously providing the infrastructure and the facilities. My thanks also go to PD Dr. Hanspeter Landolt for making himself available as a co-referee.

I greatly appreciate the moral support of my family and my friends during the last four years. My most tender thanks go to my long-standing life partner Daniel Hulliger who has always believed in me and met my complaints with cheering-up calmness.

This thesis was funded from grants of the Swiss National Foundation (START 3100-055385.98 and 3130-054991.98 to Chrstian Cajochen) and the Velux Foundation (Switzerland).