Thermophysiology and Sleep

A COMPARISON BETWEEN WOMEN WITH AND WITHOUT VASCULAR DYSREGULATION AND DIFFICULTIES INITIATING SLEEP

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Dekan

'Somne, quies rerum, placidissime, Somne, deorum,
pax animi, quem cura fugit, qui corpora duris
fessa ministeriis mulces reparasque labori'

Ovid, Metamorphoses, Bk XI: 623-625

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SUMMARY

Temperature and sleep are closely interrelated. The fall of core body temperature (CBT) at the end of the waking period is caused by heat loss via distal vasodilatation, (warm hands and feet). This process induces sleepiness. The opposite takes place at the end of the sleep episode when heat production is dominant over heat loss: distal vasoconstriction and consequently a CBT increase occur leading to an increase in the propensity to wake up. Certain individuals, mostly women, experience unusual cold thermal discomfort from cold extremities throughout their daily life. They are diagnosed as suffering from a primary vascular dysregulation (VD). VD is associated with difficulties initiating sleep (DIS), hence manifest prolonged sleep onset latency (SOL). This is possibly related to vasoconstricted distal skin regions before habitual bedtimes.

The general aim of this thesis was to obtain deeper insights into the relationship between thermoregulation and sleep. Individuals with VD and DIS provide a “model of nature” to study this relationship.

A higher vasoconstriction level at habitual bedtimes, i.e. a lower distal-proximal temperature gradient (DPG), can be caused by: (1) a circadian phase delay of the thermoregulatory system; (2) a larger circadian amplitude of DPG; or (3) a generally lower 24-h mean level of DPG. Therefore a first study was designed aiming at a chronobiological characterization of women with VD and DIS (WVD) by means of a constant routine protocol comprising an episode of 40-h total sleep deprivation (SD) after and before an 8-h sleep episode. Compared with a similar young group of women who do not have VD and DIS (CON), WVD showed no differences in habitual bed times, but a 1-h circadian phase delay of the circadian patterns of CBT, DPG, melatonin and sleepiness (Chapter 2). Sleep deprivation had no effect on the thermoregulatory system in either WVD or CON. The difference in internal phase of entrainment (ψint) could be a cause of DIS, i.e. could impact sleep onset.

Centered on the analysis of sleep stage and electroencephalogram (EEG) power spectral analysis, Chapter 3 focussed on whether the sleep architecture of WVD and CON varies and whether the challenge of SD impacts sleep of WVD and CON differently. WVD exhibited a diminished first Non-Rapid-Eye-Movement sleep (NREMS) episode, and hence reduced duration of the first NREM-REM sleep cycle. They also manifested a different evolution of delta power density (EEG power density

in the 0.5 - 2.0 Hz range) across successive NREM-REM sleep cycles, i.e. the decrease in delta-power was less pronounced from the first to the second cycle. EEG power density in the delta and alpha frequency range (0.5 - 2.0 Hz and 7.25 - 9.75 Hz, respectively) tended to be lower in WVD compared to CON. A change in internal phase of entrainment (i.e. phase delayed thermoregulatory heat loss with respect to the sleep-wake cycle) may influence not only SOL but also ultradian sleep patterns.

The second study aimed at disclosing effects of a temperature stimulus on sleep, simulating in WVD and CON reinforced heat retention and heat loss by means of cool (28℃) and warm (39℃) 35-min head-out water i mmersions, respectively, together with a neutral (35℃) bathing condition (C hapter 4). These conditions resemble the thermoregulatory effects of the falling and rising limbs of the CBT in the evening and morning, respectively. A subsequent 2-h afternoon nap revealed in CON that bathing at those temperatures in the afternoon decreases and increases convective body heat loss via the distal skin regions, prolonging and shortening SOL in a subsequent sleep episode, respectively, without affecting REM sleep (REMS) , SWS, slow-wave activity (SWA; EEG power density in the 0.5 - 4.5 Hz range), and REMS onset latency (REML). In contrast, the heat retention condition after cool bathing generated a shorter REML and a faster REMS accumulation in WVD compared to CON. Additionally, WVD had a longer lasting distal vasoconstriction, hence lower DPG values during the sleep episode after cool bathing and consequently a less pronounced CBT drop (afterdrop) than CON. WVD showed in general a lower EEG power density in frequency bins of the theta and alpha frequency ranges (4.5 - 9.75 Hz) irrespective of topography, i.e. frontal or occipital region, or bathing condition, indicating a trait-dependent feature. However, reduced SWA was found after cool bathing in the frontal region, a difference to CON that was no longer detectable in the occipital region and after warm bathing, indicating SWA as a state (temperature)-dependent characteristic in WVD. Reinforced heat retention in WVD accentuates alterations of sleep parameters already existing under normal night sleep conditions, and this indicates that at least some sleep parameters in WVD may be influenced by the different thermophysiological conditions in these individuals compared to CON.

Summarized together, the observed variations of thermoregulatory and circadian processes in WVD compared to CON are not fully reflected in the sleep EEG. The changes in these parameters are not directly related to changes in sleep stages and EEG power density.

CHAPTER 1

INTRODUCTION

The circadian system: general properties

Due to changes like the daily light-dark rhythm or seasonal rhythms, oscillator systems have emerged in living organisms as the central controllers of these rhythms, making them independent of direct environmental fluctuations in such away that anticipation of changes in the environment became possible and enabled them to predict and prepare for daily demands. In higher forms of life complicated networks of coupled oscillators driven by pacemakers evolved. These networks then became tuned to the environment by the use of synchronizing signals, called 'Zeitgebers' (e.g. light). Natural selection has favored endogenous circadian rhythmicity that, in the absence of periodic synchronizing cues from the environment, persists with an intrinsic period length (τ) close to 24 hours (hence 'circadian', from Latin circa = about and dies = day) (49, 215) in nearly all living organisms including prokaryotes (110). In mammals, circadian rhythms are generated by a master circadian pacemaker located in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus (146) (185). Circadian rhythms can be observed in a variety of physiological and behavioral variables, such as CBT, heart rate, plasma hormone levels, enzyme activity, performance, subjective alertness, or sleep-wake behavior. Light is the major zeitgeber for synchronization of the human circadian clock. Photic inputs are perceived by the retina and transmitted in the form of electrical signals directly to SCN neurons via the retino-hypothalamic tract (100, 147). In this way entrainment is achieved by daily phase-shifting, which refers to the ability of the circadian clock to alter its phase in response to appropriate stimuli. Subjects exposed to light in the early morning (before usual awakening) will exhibit an advance in their circadian phase during subsequent cycles, while light exposure during early subjective night will cause a phase delay. Light exposure during the subjective day (when exposure to light is normally expected) has little or no effect on the phase of the circadian rhythm.

During the last decade, the understanding of the cellular and molecular mechanisms of circadian rhythms has progressed (for review see (126)) and genes driving molecular circadian oscillations, so called 'clock genes' have been identified (5, 111,

126, 158, 168, 177). Recent findings have revealed that clock genes are not only expressed in the SCN but additionally in extra-SCN tissues (109, 178). According to current concepts, the master clock in the mammalian SCN synchronizes a variety of oscillators in peripheral tissues, such as liver, heart, skeletal muscles, and kidney and in this way the entire timing system can be adapted to the physiological needs of the organism. These peripheral clocks can be entrained by nonphotic cues appropriate to their function, for example feeding for the liver or activity for muscle (54, 140, 188). Although light is the strongest agent known to influence the phase of the circadian clock, other agents or stimuli can induce phase shifts of the circadian rhythm: Activity/exercise (21), food intake (54, 188), social contact (210) (pp.339-352), exogenous melatonin (10), temperature (167, 170) and drugs (99).

Disclosing the endogenous circadian rhythm

During the evolution from unicellular organisms into multicellular ones, direct and immediate (and often unpredictable) effects of the environment were also present and these effects altered the shape and function of the rhythms driven by the oscillator system. These changes became known as 'masking effects'. Accordingly, overt circadian rhythms were considered to be a mixture of endogenous components induced by the oscillator(s) – the body clock – and exogenous, or masking, components resulting from a direct effect of the environment (e.g. light, darkness). Not only external changes may mask the rhythm of the oscillator but also effects from the behavior of the organism itself upon its rhythms (sleep, activity, food intake), or the effects of physiological and biochemical processes taking place during homeostatic regulation (14, 211). Because overt rhythms might not reflect the internal oscillator exactly, attempts have been made to overcome this deficiency, and to demonstrate the endogenous nature of the observed rhythmicity by overcoming the masking effects. One method is the constant routine (CR) (for review see (75)) . The method of the CR was developed for this purpose, initially by Mills et al. (142) and later expanded (48, 120). In the latter protocol, the subjects remain awake in bed in a semirecumbant posture for 40 h, reducing the major sources of masking: motor activity is limited, no posture change, constant dim light, constant ambient temperature and humidity, sleep is forbidden, and food and drink are given in small isocaloric portions at equal intervals. Two output rhythms of the SCN are commonly used as measures of the circadian clock in humans: rhythms of CBT and melatonin (25).

Sleep, sleep EEG, and analysis of the sleep EEG

The cyclic repetition of sleep and wakefulness is essential to the basic functioning of all higher animals, including humans. But the question why the brain or body requires periodic episodes of sleep to function effectively during wakefulness still lacks a definite scientific answer despite the rapidly increasing understanding of the processes generating and maintaining sleep (181). There are several suggestions to answer the question 'why we sleep'. Among the most prominent and nonmutually exclusive are the hypotheses of energy conservation (through maximizing energy savings by reducing body and brain energy consumption) (28) (pp. 41-52), memory consolidation (sleep for brain plasticity, learning and memory) (130, 203), or metabolic processes (162, 182).

As a consequence of its still unknown function, sleep can only be defined operationally and only information about the questions of 'how we sleep' and 'how sleep is regulated' can be obtained. Human sleep can be defined on the basis of an individual's observed behavior and accompanying physiologic changes in the electrical activity of the brain. On the behavioral level, sleep is a state characterized by stereotypic posture, closed eyes, muscle relaxation, reduced responsiveness to stimuli, and reversibility. However, some of these characteristics can also be observed during wakefulness (e.g. resting with eyes closed). The development of adequate techniques of recording electrical brain activity by scalp electrodes in the late 1920s (27) led to the discovery of specific changes during sleep. Thus neuronal activity patterns fundamentally differ between sleep and wakefulness on the level of brain activity and undergo substantial changes also within sleep itself. As the understanding of the neurobiology of sleep increases, sleep is no longer viewed as a passive state (i.e. sleep as merely the absence of wakefulness). The study of the sleep EEG has revealed that sleep is not a unitary state, but a dynamic process that is actively regulated.

Sleep EEG, sleep stages, and spectral analysis

The 'gold standard' to characterize sleep is polysomnography which simultaneously records the three physiologic measures that define the main stages of sleep and wakefulness. These measures include muscle tone, recorded through electromyogram (EMG); eye movements, recorded through electrooculogram (EOG); and brain activity, recorded through EEG. A systematic method for visually scoring

human EEG was developed to ensure standardized terminology (103, 165). It is applied to divide the sleep episode into three major vigilance states: waking, rapid-eye-movement sleep (REMS), and non-rapid-eye-movement sleep (NREMS). NREMS is further subdivided into the four stages: 1, 2, 3, and 4, whereas stages 3 and 4 are often grouped together under the term 'slow wave sleep' (SWS), reflecting the occurrence of low-frequency waves (0.75-4.5Hz) which in turn, are an expression of underlying cortical synchrony (7). The scoring rules for wakefulness and NREMS are mainly based on the frequency, amplitude, and waveform of the EEG waves (Figure 1). Wakefulness is characterized by low-amplitude, high-frequency activity, while during NREMS, high-amplitude, low-frequency waves predominate. The determination of REMS additionally demands beside the appearance of a mixed frequency EEG, which looks similar to the waking EEG, low muscle tone in the submental EMG and rapid eye movements in the EOG (20). REMS can further be subdivided into two stages: tonic and phasic. The tonic stage is continuous and shows muscle atonia and random-appearing wave pattern as the main two features. Superimposed on the tonic stage of REM are intermittent phasic events which include bursts of rapid eye movements and irregularities of respiration and heart rate.

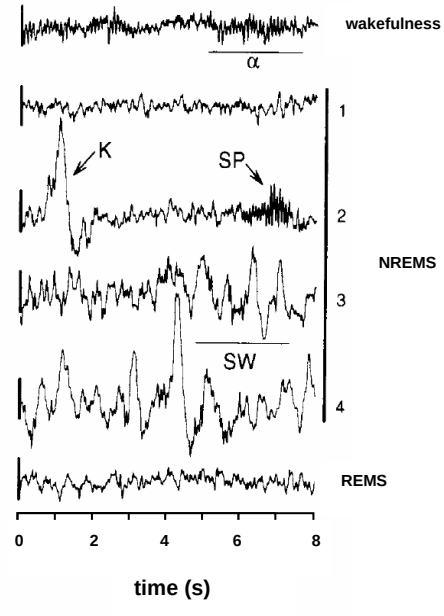


Figure 1. *Eight second EEG tracings obtained during wakefulness, NREMS (stages 1 to 4) and REMS. Wakefulness is characterized by alpha activity (α; 8-11 Hz). K-complexes (K) and sleep spindles (SP; 12-14 Hz) occur preferentially in stage 2. Slow waves (SW; 0.75-4.5 Hz) are predominant in stage 3 and 4. REMS shows a dominant theta activity (4-8 Hz). Bars on the left of each trace*

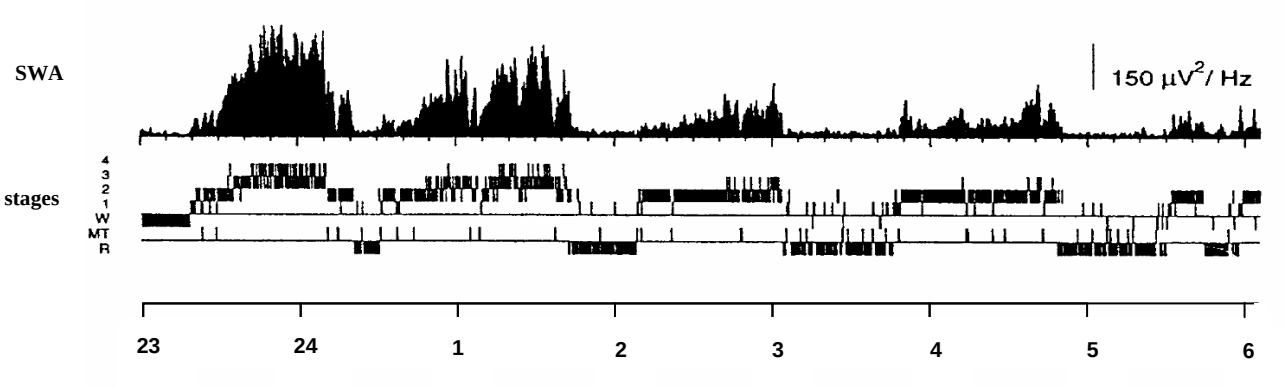


***Figure 2**. Sleep stages and time course of EEG slow-wave activity (SWA, mean power density in the 0.75 - 4.5 Hz range) during an 8-h sleep episode. R=REMS; MT=movement time; W=waking.*

Continuous recording of the sleep EEG reveals that sleep stages do not occur at random but rather appear in cyclic manner (58). In general, after its onset, sleep starts with an episode of NREMS lasting 60-90 min before the first REMS episode occurs. This cycle is repeated four to five times during a normal 8-h sleep episode (58). The interval from the beginning of one NREMS period to the beginning of the next is used for defining a sleep cycle (80). The duration of the NREMS-REMS cycle is generally about 90 min but may vary from 60 to 120 min (81). The proportion of different sleep stages in a sleep cycle changes across the sleep episode in such a way, that the percentage of SWS is highest in the first sleep cycle and diminishes over subsequent cycles, whereas the percentage of REMS and stage 2 sleep increases from the first to the last cycle (Figure 2). Approximately 75 to 80 % and 20 to 25% of total sleep time is usually spent in NREMS and REMS, respectively (132).

Synchronization of brain activity, i.e. the simultaneous activation of large population of neurons, is a fundamental feature that discriminates NREMS from REMS and wakefulness, at least until the frequency of 30 Hz (high-frequency synchronous activity also emerges during REMS in the 30-80 Hz range) (101). The EEG during wakefulness and REMS is similar, and exhibits low spatio-temporal coherence in the cerebral cortex. In contrast, the high-amplitude, low-frequency activity during NREMS is synchronized over large cortical areas (59). Two essential types of synchronized oscillations during NREMS are slow waves and spindles (Figure 1). Sleep spindles are transient (0.5-2 s) oscillations of about 12-15 Hz that recur approximately every 3-10 s. Their name "spindle" refers to their characteristic shape with progressively increasing, then decreasing amplitude. Sleep spindles are more abundant in stage 2 than SWS (55, 68, 218).

Quantifying the EEG by visual scoring has its limitations because the sleep stages are based on rather arbitrary, discrete criteria. This does not properly reflect the continuous physiological mechanisms that underlie changes in EEG. And the patterns of fast EEG activity are invisible to the visual scorer because of their low amplitude. Therefore, other methods have been developed by which the EEG signal can be analyzed. One method to quantify EEG activity is the spectral analysis of the sleep EEG using the fast Fourier transform (FFT) (47). Shortly after FFT was developed it was applied to the EEG by Dumermuth and Flühler (76). Briefly, the FFT decomposes a waveform (e.g. EEG waves) into sinusoids of different frequency and phase which sum the original waveform. It identifies or distinguishes the different frequency sinusoids and their respective amplitudes. Thereby the EEG signal is transformed from a time into a frequency domain. This requires a stationary signal. EEG waves during sleep are not stationary, but by analyzing short time windows (e.g. 4 s), a quasi-stationary signal can be obtained for these short intervals. The length of the time window determines the slowest detectable wave and with it the frequency resolution. For the short time window, spectral analysis calculates overall power density per frequency bin (i.e. $\mu V^2/Hz$) by combining incidence and amplitude. The resulting power spectrum depicts this power as a function of frequency bin and thus expresses the contribution of each frequency bin to the power of the total signal. Power density in the frequencies between 0.75-4.5 Hz (slow-wave activity, SWA) is commonly used to quantify slow EEG activity. SWA shows a gradual increase at the beginning of the NREMS episodes until a plateau level is reached and then shortly before the REMS episode falls to low levels (Figure 2). In addition a global declining trend of SWA over a sleep episode is present. This SWA pattern is only roughly reflected by the stepwise function of the visual scoring procedure.

Circadian, homeostatic, and ultradian regulation of the sleep-wake cycle

Three major processes underlie sleep regulation (Figure 3): a homeostatic process responsible for the rise of sleep propensity during waking and its dissipation during sleep, a circadian process that is basically independent of prior sleep and waking, and is responsible for the alternation of periods with high and low sleep propensity, and an ultradian process occurring within the sleep episode representing the alternation of the two basic sleep states NREMS and REMS.

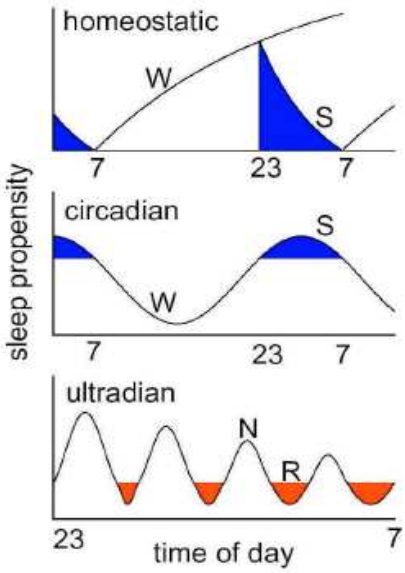


Figure 3. *Schematic representation of the three major processes underlying sleep regulation. W, waking; S, sleep; N, non-REM sleep; R, REM sleep. The progressive decline on non-REM Intensity is represented both in the top and bottom diagrams (decline of ultradian amplitude). The increase in the duration of successive REM sleep episodes is indicated(Figure from (2))*

Various models were used to specify the processes involved in the regulation of sleep (2) and to analyze experimental data. Among those models, the two-process model of sleep regulation has been evaluated and confirmed most frequently and has been able to simulate and predict sleep behavior in different physiological and experimental conditions. The two-process model of sleep regulation (33, 53) addresses the homeostatic and circadian aspects of sleep regulation. It assumes an interaction of its two constituent processes, the homeostatic Process S and the circadian Process C. The level of the sleep-wake-dependent Process S rises during waking and declines during sleep. Process C, in contrast, is totally controlled by the circadian pacemaker, is independent of sleep and waking, and is proposed to set the upper and lower limits to Process S, therefore determining the onset (if S reaches the upper limit during waking) and termination (if S reaches the lower limit during sleep) of a sleep episode, respectively (53). Therefore Process S is the regulated variable. It is controlled by Process C and by other influences such as conscious decisions to stay awake (or to wake up), or by influences as pain, social interactions or noise. As the phase of Process C is controlled by light exposure to the retina, the timing of sleep and waking, i.e. the sleep-wake behavior indirectly influences Process C.

As closed eyelids reduce light intensities falling on the retina (8), sleep modifies retinal light input and, thereby, the phase of the circadian pacemaker. Certain behavior as going to bed late, leads to delays of the circadian pacemaker which reinforces itself and may lead to large differences in the phase angle between sleep and the light-dark cycle. Process C not only affects Process S but also the ultradian process or REMS oscillator. The interaction of these three major processes underlying sleep regulation can be summarized as follows: The interaction of

Process C and S in the sleep homeostat determines the timing and intensity of sleep (53). The sleep architecture results from the sleep homeostat in which, due to input of the REMS oscillator, the ultradian pattern of SWA and REMS is generated. In the following, each of the three sleep regulation of the sleep-wake cycle will be first described separately and then integrated.

Homeostatic regulation of the sleep-wake rhythm

A homeostatic pressure for sleep progressively builds up during waking and dissipates during the following sleep episode. The extent of homeostatic sleep pressure directly depends on the duration of prior wakefulness (34, 61). For example a prolongation of the waking time prior to sleep is followed by a decrease in the latency to sleep onset (38).

A reliable marker for homeostatic sleep pressure has been emerged to be SWA during NREMS, since this metric correlates with sleep need and increased wakefulness preceding sleep (61, 65). It decreases throughout the course of the sleep episode, almost independent of time of day, i.e. of circadian phase (69, 207), and is augmented at the beginning of the night when wakefulness prior to sleep has been extended (34, 68). Conversely reduction of sleep pressure by an early evening nap results in reduced SWA at the beginning of the subsequent night sleep (209). Thus NREMS pressure indexed by SWA appears to be regulated by a homeostatic process which keeps track of the prior history of sleep and wakefulness.

Also REMS is - beside the markedly circadian control - under homeostatic control. Although an intensity dimension has not been identified, a deficit appears to be compensated by an increase in REMS duration (38, 57).

However, gaps remain in the understanding of the neurobiological basis of the homeostatic process S. Process S is thought to represent the need for sleep, therefore attempts were made to find a physiological correlate for sleep need. It has been suggested that one mechanism for homeostatic sleep drive might be an accumulation of a sleep-promoting substance that enhances the activity of sleep-promoting cells and reduces the activity of wake-promoting neurons (23, 24, 162, 189).

Unlike the quite precise localization and mechanisms of the circadian rhythm generator (148), the localization of and the mechanisms for possible homeostatic determinants have not been conclusively identified. It may eventually be found that

this system is widely distributed in the central nervous system, contributed to by multitudinous neuronal and hormonal substances.

Circadian regulation of the sleep-wake rhythm

Many sleep parameters such as sleep propensity (measured by speed falling asleep or duration of sleep), sleep timing, sleep structure and the consolidation of sleep and wakefulness are strongly influenced by the endogenous circadian pacemaker (65). This has been demonstrated in studies where SCN lesions abolish the near 24-h periodicity of sleep-wake propensity in both humans (46) and other primates (78) and result in an uniform distribution of short sleep and wake bouts across the 24-h day. It has been shown that SCN neuronal activity is responsive to changes in sleep and EEG SWA (56).

The SCN is thought to generate a wake signal that increases in strength throughout the habitual wake episode, peaking in the evening hours at ~2200 h, i.e. around habitual bedtime. The strength of this signal declines during the habitual sleep episode to reach a minimum at ~0600 h which coincides with the CBT nadir and is near the usual time of awakening (63). In the absence of this wake signal, sleep-wake cycle consolidation is lost and the monophasic sleep-wake cycle is replaced by a polyphasic sleep-wake cycle, presumably dictated primarily by sleep homeostasis. A consolidated 8-h episode of sleep can only be obtained at one specific phase relationship between the sleep-wake cycle and endogenous circadian rhythmicity, which means, only when sleep is initiated ~6 h before the temperature nadir, i.e. shortly after the crest of the wake propensity rhythm, sleep will remain virtually uninterrupted for 8 h. Thus under entrained conditions, the circadian drive to initiate and maintain sleep is low at the habitual bedtime and high at the habitual wake time (208). It was hypothesized that this paradoxical circadian timing of the circadian drive for waking (indexed by latency to sleep onset, sleep efficiency, and subjective alertness) may counteract the increase in sleep propensity and allows to maintain a consolidated 16-h episode of wakefulness each day. And likewise the increase in circadian sleep propensity that occurs as the night progresses counteracts the decrease in sleep propensity associated with accumulated sleep and allows to maintain a consolidated 8-h sleep episode (66). Not only sleep timing, but also internal sleep structure depends on circadian phase. REMS undergoes a strong circadian modulation with a maximum of REMS propensity in the morning hours shortly after the minimum of the circadian rhythm of CBT (52, 79) which under normal

entrained condition is located about 1.5 h prior to awakening (50). Within NREMS both SWA and sleep spindles are affected by circadian factors and show an endogenous circadian rhythm (65).

There are clear ideas about the location in the brain where the interactions between Process S and Process C occur (174) and additionally, a molecular basis for this interaction has been proposed (125, 213). Taken separately, both, Process S and Process C alone would allow for some extent of consolidation of wake and sleep episodes. Per se they do not depend on each other, they operate independently (62, 144, 194). However, only when taken together at the normal phase relationship do these biologic processes allow for the expression of a sustained bout of 14 to 18 hours of relatively stable wakefulness and a similarly stable 6 to 9 hour bout of consolidated sleep in the adult human (66).

Ultradian regulation of sleep

There are two models concerning the ultradian NREM-REM sleep cycle: the 2-process model of Borbély (33) and the reciprocal interaction model of REM regulation of McCarley and Hobson (102, 133). The latter can be summarized as follows: REM inhibiting neurons (aminergic RemOff cells) have an inhibitory auto feedback that stops their own activity and allows other neurons (cholinergic RemOn cells) to gain activity and generate REMS evident from the 80-120 min periodicity of the NREM-REM sleep cycle in humans. Under normal entrained conditions the percentage of NREMS within NREM/REM sleep cycles tends to decrease whereas the percentage of REMS tends to increase toward the end of the night. It has been proposed in a model of sleep regulation (1) that sleep architecture results from the sleep homeostat in which, due to the input of the REMS oscillator, the ultradian pattern of SWA and REMS is generated. It has been shown (124) that monoamine oxidase inhibitors are capable of abolishing REMS in humans but do not influence the natural course of SWA in the absence of normal NREM-REM sleep cycles (i.e. SWA rises after sleep onset and then declines exponentially). Therefore, the shape of the SWA curve is consistent with process S in the two-process model. REMS, in contrast, could reflect an ultradian process which interrupts or inhibits the basic mechanism underlying process S with a period of 80 to 120 minutes. However, the neural mechanisms of an underlying ultradian oscillator of REMS have been intensively studied (158) but not yet identified.

Genetic aspect of sleep-wake regulation: clinical impact

The clock-genes involved in the basic molecular mechanism behind circadian rhythms are essential for circadian timekeeping as demonstrated by the study of deletion or mutation of these genes leading to dramatic alterations of circadian period or complete arrhythmicity in rodents (44, 109). In humans genetic analysis has shown that clock gene variations are involved in the development of certain types of circadian rhythm sleep disorders such as the advanced sleep phase syndrome (77, 158) or the delayed sleep phase syndrome (77, 96, 158).

Circadian rhythm sleep disorders

As mentioned above, only at a specific phase relationship between the sleep-wake cycle and endogenous circadian rhythmicity a consolidated sleep and wake episode is assured. Circadian rhythm sleep disorders arise from disruption of the circadian timing system or a misalignment between the endogenous circadian timing and the external 24-h social and physical environment resulting in complaints of insomnia and/or excessive sleepiness and impairment in important areas of functioning and quality of life. Circadian rhythm sleep disorders can be persistent like the delayed sleep phase syndrome (DSPS) or the advanced sleep phase syndrome (ASPS), or periodic like the non-24-hour sleep-wake disorder (free-running type), or transient and behaviorally induced, respectively, like the jet lag syndrome or the shift-work sleep disorder (22, 123).

DSPS is characterized by sleep times that are delayed three to six hours relative to the desired or socially acceptable sleep-wake schedules (32, 206). Subjects with DSPS show delayed circadian CBT, melatonin, and cortisol rhythms relative to their sleep-wake cycle, i.e. they have an altered phase relationship between those rhythms compared to control normal subjects (180, 195, 204). The sleep characteristics of DSPS are prolonged sleep onset latencies, increased wake time after sleep onset and consequently poor sleep efficiency, and therefore, due to the need to wake for social or work commitments, reduced amount of sleep. ASPS in contrast is characterized by early evening sleepiness, an early sleep onset and morning awakening earlier than desired even if the person attempts to delay bedtime significantly. Circadian CBT and melatonin rhythms have been shown to be advanced compared with control normal sleepers (122).

The non-24-hour sleep-wake disorder is observed mainly in blind people without light perception (171, 184) and therefore it is thought to be related to disruption of input pathways, keeping light-dark information from reaching the SCN (108). This free-running circadian rhythm is characterized by periods of good sleep (i.e. long duration sleep, no daytime napping) when the endogenous pacemaker is in phase with sleep times and periods of poor sleep (i.e. short duration sleep with daytime naps) when the endogenous circadian rhythm is not in phase with the conventional sleep and wake times (184). This pattern is due to the steady daily drift of the major sleep and wake times due to the presumed cut off from the light-dark cycle. And because the endogenous circadian period in humans is usually slightly longer than 24 hours, patients will report a progressive delay in the timing of sleep and wake times.

Jet lag as a transient circadian rhythm disorder is experienced by travelers and flight crews as they cross several time zones in a short period of time and characterized by a desynchrony between the endogenous circadian rhythm (still timed to their home environment) and the clock time of the new environment. The symptoms of jet lag due to this desynchrony include difficulties in initiating and maintaining sleep, poor daytime functioning due to sleepiness, impaired alertness and performance (205). For the sleep-wake temperature and other hormonal rhythms, it can take about 1.5 days/h of adjustment (121). The term 'social jetlag' has been introduced as a circadian sleep disorder of subjects featuring late chronotype (214). They show the largest differences in sleep timing between work and free days leading to a considerable sleep debt on work days, for which they compensate on free days. The discrepancy between work and free days, between social and biological time, was therefore described as 'social jetlag'. Another self-imposed internal desynchronization between the endogenous circadian rhythms and sleep occurs chronically during shift-work (22), resulting in complaints of unrefreshing sleep, insomnia during the daytime and excessive sleepiness during the nocturnal work time hours, that vary depending on the work schedule (73).

As sleep complaints, especially difficulties in initiating sleep, are often reported in circadian rhythm disorders, it might be possible that typical insomnia - which is not diagnosed as a specific circadian rhythm sleep disorder - could be associated with alterations in the circadian oscillator (150). People complaining about sleep disturbances should additionally be examined for a possible circadian dysbalance before applying to early unspecific pharmacological interventions.

Thermoregulation

When the sleep-wake cycle is synchronized with the geophysical light-dark cycle, CBT exhibits a circadian rhythm with a temperature maximum in the early evening and a minimum in the second half of the nocturnal sleep episode (Figure 4). This rhythm is generated and modulated through homeostatic and circadian processes and independent of whether a subject is allowed to sleep or not during the subjective biological night as demonstrated in studies performed under the very stringent protocol of a CR (120). In the following, the homeostatic and the circadian regulation of CBT are explained separately. In order to understand circadian regulation of the CBT rhythm it is important to describe first how CBT is homeostatically regulated.

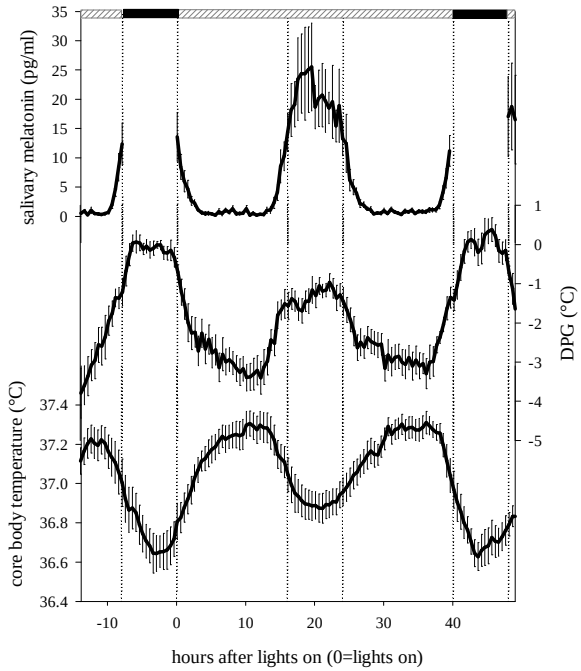
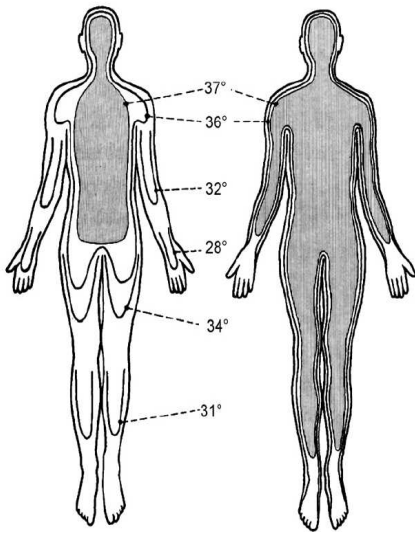


Figure 4*. Circadian core body temperature, distal-proximal skin temperature gradient (DPG), and melatonin waveforms for 9 healthy young women. Black bar on the top, time of scheduled sleep episode. Data were plotted with respect to scheduled wake time (scheduled wake time = 0). Mean ±SE is shown.*

Homeostatic regulation of CBT

Homeostasis in general denotes the relative constancy of physico-chemical properties of the internal environment of an organism as being maintained by regulation (141). Regarding temperature regulation the term of homeothermy is used, meaning the pattern of temperature regulation in tachymetabolic ('warm-blooded') species in which the cyclic (e.g. nycthemerally) variation in CBT is maintained within arbitrarily defined limits despite much larger variations in ambient temperatures (141). There is substantial evidence indicating that homeostatic control of CBT is mediated by a hierarchically organized set of neuronal mechanisms, with the preoptic area of the hypothalamus (POAH) at the top of the hierarchy as the predominant integrator of thermal information from central and peripheral parts of the body (145, 176). In addition to the homeostatic principle rostral projection from the circadian pacemaker,

i.e. the SCN, to the preoptic area serves the circadian modulation of CBT (145, 176). Nocturnal secretion of the pineal hormone melatonin, which is under control of the SCN, plays an additional role in the endogenous regulation of CBT in the evening (42). In humans melatonin follows the inverse time course of the CBT rhythm and is, at least partially, involved in the regulation of its circadian amplitude (41). Additionally melatonin may play a role in the control of the skin blood flow inducing distal vasodilatation, hence heat loss and decrease of CBT (4, 9). Under resting conditions thermoregulation in humans consists of two compartments, the heat-producing core and the heat-loss regulating shell (19) (Figure 5).



cool room temperature (20°C)
or
standing
awake

warm room temperature (35°C)
or
lying down
asleep

Figure 5. *The schematic diagram shows a human body in a cool (20°C) and warm (35°C) environment. Similar distributions of the core and skin temperatures can be observed under waking and standing conditions (cool) and, under sleeping and lying down conditions (warm) in a thermoneutral environment (Figure from (15) p.43-112)*

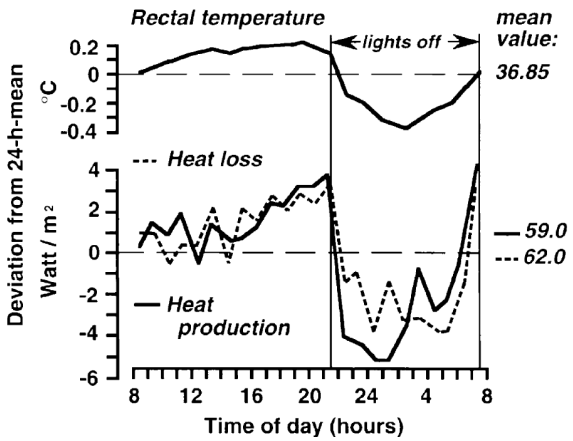


Figure 6*. Circadian patterns of core body temperature (CBT), heat production and heat loss (Figure from (114). CBT declines when heat loss surpasses heat production.*

To regulate the endogenous circadian CBT rhythm changes in shell size take place. Distal skin temperature rises in the evening whereas proximal skin temperature, heat production, and CBT decline - and this pattern switches in the morning (18, 115). The core is homeostatically maintained within the narrow range of temperatures bound by the core threshold temperatures for heat production (shivering) and heat loss (sweating) defined as the interthreshold zone around 37℃. In the range of this zone responses of metabolic heat production and evaporative heat loss are absent and maintenance of CBT is achieved solely by behavioral adjustments of heat loss and heat gain and by vasomotor responses assisting in either heat loss or heat retention i.e. vasodilatation or vasoconstriction. This concept suggests that CBT is not regulated at a precise level but its regulation is coarse allowing CBT under normal physiological conditions to fluctuate within the interthreshold zone and only larger fluctuations in CBT also activate the autonomic responses of either shivering or sweating; in contrast the shell is rather poikilothermic and therefore largely dependent on environmental conditions (19) (Figure 5).

In the cold the shell is large, in a warm environment the shell is small (Figure 5). The regulation of blood vessel diameters occurs very rapidly before CBT has enough time to change. This so-called feed-forward regulation with respect to CBT is an important property of the thermophysiological 'core/shell' principle (12, 16). Shell size is autonomically regulated via constriction or dilatation of peripheral blood vessels, mainly of smooth muscles in arterioles and additionally in distal skin regions of smooth muscles in arteriovenous anastomoses (AVAs) (16, 94). AVAs are shunts between arterioles and venules exclusively found in distal skin regions (92). When they are open, blood loaded with heat flows about 10,000 times faster than via capillary blood flow (94, 95) and directly from arterioles to the dermal venous plexus enabling an efficient heat exchange. Sympathetic nerve activity is therefore crucial for the regulation of the peripheral vascular system.

The exact neural process by which the stability of the body temperature is achieved is still a matter of debate.

One hypothesis is that the observed thermoregulatory responses to cold and warm stimuli could be an indication of a deviation from a stable reference signal ('set-point'). That is, activation of heat combating responses (e.g. vasodilatation, hot feeling, preference of cold environment, sweating) means that CBT is above the reference temperature and vice versa: activation of cold combating responses (e.g.

skin vasoconstriction, piloerection, increased thermogenesis, feeling of cold, shivering) means that CBT is below the reference temperature (35). Another hypothesis is the reciprocal cross inhibition model (RCI) (29, 30, 139). According to the RCI, thermo afferent information from peripheral and core sensors provides the neural drive for heat production (shivering) and heat loss (sweating). The excitatory drive in the heat production sensor-to-effector pathway also provides an inhibitory drive in heat loss sensor-to-effector pathway and vice versa. In this manner the overlapping activity/temperature characteristics of the cold and warm preoptic sensors can establish a regulated level of CBT simply by the reciprocal inhibition of the heat production and heat loss pathways. Therefore, in contrast to the set-point theory, in the RCI model there is no stable reference signal generator. But the RCI creates temperature stabilization at a temperature zone at which neither metabolic heat production nor evaporative heat loss effectors were active. The feature of this system of regulation is not the comparison of a variable with a constant but is the interplay of two variables with different response coefficients (30).

Circadian regulation of CBT

Whereas the POAH is necessary for optimal maintenance of homeostasis in CBT the SCN are the primary pacemaker for the circadian CBT rhythm. And whereas thermal homeostasis described above enables the body to respond appropriately to acute changes in the environment it has also a temporal aspect that facilitates the prediction of environmental challenges and thereby allowing corrective responses to such challenges to occur in advance. This predictive homeostasis is represented in the thermal physiology of the circadian system. But the precise interaction of these brain regions in generating and maintaining a circadian CBT rhythm is still unclear, despite the reported projections from the SCN to the POAH which may influence CBT and the sleep-wake cycle (45, 60, 145). Generally CBT is regulated by a balance between heat production and heat loss; this is specifically the case for the circadian rhythm in CBT (Figure 6) (17, 18). Heat production underlies a circadian rhythm, which is phase advanced by ca. 1 hour in comparison to the circadian rhythm in heat loss (i.e. when heat production surpasses heat loss CBT increases (Figure 6)). This phase angle difference determines the circadian amplitude of ca. 0.4℃ in CBT (13, 17). However, the circadian patterns of heat loss and heat production are not uniform sine waves with a 24-h period: Heat loss in the evening is more dominant than the reduction in heat production, and in the morning the reverse is true (Figure

6) (17, 18, 120). The SCN influences the circadian rhythm of skin temperature and CBT, respectively, but it has also been argued that reciprocally body temperatures may also affect the SCN (198).

There is a still unfinished old discussion as to how the circadian system interacts with the thermoregulatory system (11, 40, 166). Aschoff generally assumed that CBT is primarily under homeostatic control and is secondarily modulated by the circadian system through daily oscillation in the thermoregulatory 'set-point' implying the overt daily rhythm of CBT being the result of the concerted action of circadian and homeostatic process (11, 40, 166). Whereas others hypothesize that the homeostatic control and the circadian control of thermoregulation are not integrated but that the thermoregulatory system and the circadian system have independent control of the effector organs that regulate heat production and heat dissipation without a modulation of the 'set-point' (166).

Thermoregulation and sleep

Early studies in humans revealed a close temporal relationship between sleep onset and the CBT (12, 51, 219). Sleep is typically initiated on the declining portion of the CBT curve when its rate of change and body heat loss is maximal (43, 151, 219). It has been demonstrated that the circadian rhythm of sleep propensity (or the ability to fall asleep) is more closely related to body heat loss than CBT itself (117). The concept that it is body heat loss which is crucial for sleepiness and sleep initiation is supported by a number of studies (36, 89, 143, 196). That body temperature can play a modulatory role on sleep-regulating systems has been reported by the observation that subtle changes in skin temperature within the thermoneutral range modulate the firing properties of thermosensitive neurons in brain areas involved in sleep regulation. Such changes in skin temperature occur autonomously under control of the circadian system but also with specific behaviors and could contribute to changes in sleep propensity (197). It has been shown that induction of small changes in skin temperature affects sleep at least as strongly as the induction of small changes in core temperature (164). The temperature of the skin - rather than the core - may turn out to be the stimulus of major importance to circadian and arousal regulating systems like the sleep-wake cycle (113).

The close relationship between thermoregulation and sleep has been supported by the findings that changes in body temperatures may trigger somnogenic brain areas as the medial preoptic area (192) and the ventrolateral preoptic area (173) to initiate

sleep, either indirectly through nerve afferents activated by cold and warm receptors located in the dermis and core, or directly through changes in core blood temperature leading to changed spinal cord and brain temperatures (197).

It is known that the homeostatic sleep drive increasing with duration of being awake obviously impairs alertness and performance (65, 67, 216), but does not much influence the thermoregulatory system (67). Thus, of the two regulatory aspects of sleep, the circadian and the homeostatic (33, 53), only the former seems to be related to thermoregulation. In the following the influences of sleep on thermoregulation and vice versa are described separately.

Effects of sleep on thermoregulation

Animal studies, and a few early studies in humans provide evidence that the POAH is not only involved in CBT regulation but also in sleep-wake regulation (137, 138, 191, 202). The POAH also contains warm-sensitive neurons (136). Activation of warm-sensitive neurons by local POAH warming promotes sleep onset increases NREMS and increases EEG delta activity (138). Activation of warm-sensitive neurons in the POAH inhibits multiple arousal-related structures belonging to the reticular activating system (134-136, 138). On the other hand mild POAH cooling strongly suppresses both NREMS and REMS (135).

It has also been hypothesized that SWS is controlled by thermoregulatory mechanisms and provides brain and body cooling as a primary homeostatic feedback process (136). The warm-sensitive neurons are assumed to integrate thermoregulation and SWS control (153, 191). Taken together, the POAH together with the basal forebrain neuronal network seems to integrate thermoregulatory and hypnotic functions on a central level.

In humans, however, the evidence for a thermoregulatory role of sleep is weak. Most studies showing correlations of CBT decline with SWS have not been carried out under controlled conditions. In particular the relevance of a change in posture (lying down) has been neglected - in most studies subjects usually lie down just before lights off (91, 179). This major masking effect has confounded prior studies and renders their conclusions doubtful.

However, these 'masking' mechanisms may be of relevance under daily life conditions facilitating and fastening the sleep onset process. Namely in that the more rapid increase in heat loss due to posture change and relaxation stimulates

thermosensitive neurons in peripheral skin that innervate the POAH where thermosensitive neurons which are responsible for sleep initiation are activated. And, in turn, efferent warm-sensitive neurons in the POAH are stimulated which innervate other somnogenic brain structures while thermosensitive neurons innervating wake promoting brain areas are inhibited. The overall result is the activation of sleep promoting areas and the inhibition of wake promoting areas resulting in an increase in sleepiness which leads to sleep onset (90).

But only with constant routine (CR) conditions long before lights out do the underlying temporal correlations become visible. Immediately after lights off it has been found that distal and proximal skin temperatures increase and heart rate decreases before onset of sleep stage 2 (116). The appearance of SWS does not further influence the process of heat redistribution which had already begun with relaxation after lights off and before the onset of sleep (113). Further substantiation comes from the rebound sleep after 40 hours sleep deprivation: although SWS and slow wave activity (SWA) are markedly increased, CBT does not change (64, 67). Conversely, forced desynchrony experiments revealed that the circadian influence on SWA is small, but on CBT is strong (65). Thus sleep in humans as measured by sleep depth or low frequency power density seems not to be related to thermoregulation.

There are of course interactions between sleep and thermoregulation when considering the different *thermoregulatory responses* that depend on sleep stage when in a cool or warm environment. In contrast to SWS REMS is characterized by a suppression of the hypothalamic integration of homeostatic temperature regulation, i.e markedly inhibited thermoregulatory responses during REMS (160).

In summary, SWS in humans does not seem to have a major thermoregulatory function. REMS suppresses hypothalamic integration of homeostatic temperature regulation, but during phasic REMS sympathetic drive increases dramatically with the above described physiological consequences. The sleepiness/sleep regulatory system feeds back to the thermoregulatory system only indirectly via sleep-related behaviors (e.g. relaxation, lying down).

Thermoregulatory effects on sleep

Regarding the sleep initiating process, it is well known and described that distal vasodilatation, hence warm hands and feet, and peripheral heat loss, is strongly associated with sleepiness and sleep induction (117, 118). These temperature

changes may act as a trigger to feel sleepy and therefore increase the chance of falling asleep. In the following, thermoregulatory influences on sleep after sleep onset will be discussed.

In humans CBT (and body heat content) can be effectively manipulated by body immersion in warm or cold baths. Changes e.g. in environmental air temperature induce counter-regulatory effects of the shell which protect the core. Body immersion changes CBT directly by external uptake or liberation of heat directly via conductive heat transfer. Water offers negligible thermal insulation at the skin surface, and during cold immersion skin temperature rapidly falls towards water temperature (26). Notwithstanding this lower skin temperature more rapid conductive heat losses occur with CBT falling 2-5 times more quickly compared with that observed in air at the same temperature (187). The change in CBT follows a characteristic dynamic time course with homeostatic counteractions to get back to the pre-intervention set-point level. Some of the effects of body heat load on sleep have been measured. However, no study exists showing effects on sleep after a cold load. A 40℃ warm bath for 30 min in the evening (0.5-2h before sleep onset) could increase CBT by 1.6-2.6℃, delay the CBT minimum, shorten sleep onset latency and increase SWS in elderly female insomniacs (age > 60y) (71, 72, 107) as well as in healthy young subjects (20-33y) (39, 104-106). However, a warm bath in the morning or afternoon had no effects on night sleep (39). Another study showed that a warm full bath or footbath before sleep facilitated earlier sleep onset (190). Both manipulations elevated mean skin temperatures whereas CBT was increased (by ca. 1℃) only after a full bath together with increased sleep stage 3 and decreased REMS. Taken together, passive body heating by immersion in warm water increases SWS and decreases sleep onset latency, probably via a thermolytic mechanism. Nevertheless, no correlation was found between the amount of SWS and body cooling (6, 175) nor was SWS directly related to variations in CBT.

A close temporal relationship has been found between the circadian rhythm of REMS propensity and CBT with a narrow peak in REMS propensity located shortly after the minimum in CBT (51, 65). REML was shortest, REMS episode duration was longest and the amount of REMS was greatest around the CBT minimum (51). These findings together with those of heat load experiments, indicate that an increase in

CBT decreases REMS propensity with prolonged REML which could reflect the inhibitory relationship between NREMS and REMS propensity.

The clinical problem of difficulties initiating sleep

The prevalence of exclusively difficulties in initiating sleep (DIS) as a sleep problem more than three times a week was reported in a large population survey in western Europe to be about 10%. More than 8% complaint about sleep latencies longer than 30 minutes (156), and in a randomized Swiss urban sample 16% reported to have more than three times a week longer than 30 min to fall asleep (119). Exclusively DIS are the main symptom of sleep disturbances as the primary sleep onset insomnia (SOI) and the delayed sleep phase syndrome (DSPS). SOI is typically characterized by frequent (i.e. ≥3 nights a week) DIS with little or no difficulty in maintaining sleep once initiated. It has been reported using a constant routine protocol that subjects with exclusively SOI show a delayed circadian CBT rhythm compared to normal sleepers (150). This implicates a circadian rhythm disturbance in subjects suffering from SOI hence guiding SOI towards a secondary insomnia. Others (85) found in exclusively SOI significantly lower finger temperatures than good sleepers, while seated during a 15-minute presleep period and lying in bed from lights off to the onset of stage 2. Unfortunately, they made no measurements of CBT or melatonin. Therefore no conclusions about the circadian pattern of those SOI subjects can be drawn. An evident circadian disorder together with classical symptoms of chronic sleep-onset insomnia represents DSPS. Regarding the close relationship between high distal skin temperature and short sleep onset latency (117) it would be of interest whether these patients like subjects with SOI experience cooler finger temperatures.

Insomniacs and especially sleep onset insomniacs (98) have been reported to exhibit heightened anxiety levels and suffer from unpleasant thoughts and excessive worry during the pre-sleep period and during wakefulness (3, 97, 149). Unlike good sleepers who are generally relaxed when attempting sleep, individuals with insomnia are often not relaxed and even get anxious when retiring for bed and trying to sleep. They fear to be unable to fall asleep and to experience disturbed, unrefreshing sleep, and consequently they are under a self-pressure to fall asleep as fast as possible, resulting in a vicious circle. These worries, or the inability to relax may interfere with the normal sleep related decrease of sympathetic nervous system (SNS) activity or even produce an increased SNS activity. Additionally it has been reported that

chronic primary insomnia is characterized as a state of hyperarousal, which can be seen in various signs of peripheral and central activation and with various symptom and behavioral manifestations such as for example excessive worry (169). In this context it has been found that chronic persistent insomnia is associated with an overall hypersecretion of ACTH and cortisol (200). And in contrast to chronically stressed individuals who show high evening cortisol but lower morning cortisol, in chronic insomniacs there is an around-the-clock activation of the HPA axis. This contributes to the suggested heightened arousal in insomniacs (169). However, there are also contradictory reports (199). Other physiologic parameters providing evidence of a sympathetic nervous system hyperarousal in insomnia have been reported as increased basal metabolic rate (31), increased CBT (128), altered heart rate variability (32, 70, 74), or increased high frequency EEG activity (beta and gamma frequency range) around sleep onset, during NREMS and REMS and cortical activation on EEG (154, 155, 161). An additional complication is that hyperarousals can be physiological, emotional, cognitive, or a combination of these (127).

Considering the thermoregulatory concepts above some form of DIS may be related to vasoconstricted distal skin regions (cold hands and feet). An increased SNS activity would additionally promote distal vasoconstriction. Data of lower distal skin temperatures in SOI around sleep onset and the longer time that insomniacs can experience to reach the elevated nightly toe temperature of good sleepers support the thermophysiological link to sleep initiation (36, 85). And additionally as stated above DIS may (also) be a chronobiological disorder. If the individual's biological clock is phase delayed then sleep can only be initiated at a later time than usual. The proximate mechanism may be that the circadian rhythm of readiness to vasodilate is too late, not the ability to do so, these diagnoses need to be differentiated. Considering this it would be even more detrimental for subjects having a delay of their biological clock and additionally an inability to proper vasodilate distal skin vessels, for example because of their increased SNS activity. An epidemiological survey in Switzerland revealed that 31% of women and 7% of men between the age of 20-40 years experience symptoms of cold hands and feet and of these groups 16% and 14 % reported to have concomitant DIS (119). Vascular dysregulation was significantly associated with DIS and the most significant predictor for DIS (112).

Primary vascular dysregulation

There are some individuals who, throughout their lives, respond to stimuli such as cold, mechanical, or emotional stress with more frequent and more intense vasoconstriction (vasospasm) than the average population. Vasospasm is defined as inappropriate constriction or insufficient dilatation in the microcirculation (86, 87, 129). Highly prevalent in the Japanese population (152) where it is called 'hieshô' (冷え性) in Japanese meaning having a disposition for feeling cold. Actually it is a regulation of circulation that is not properly adapted to the local or systemic needs and can be defined as a vascular dysregulation (VD). Previously it has been called 'vasospastic syndrome' due to the observed vasoconstriction as the most prominent reaction in those subjects. Clinical criteria are used to distinguish subjects with uncomplicated or primary VD from those with secondary VD. Whereas primary VD is an inborn predisposition to respond differently to various stimuli a secondary VD is a local or systemic dysregulation as a consequence of an underlying disease including infectious, autoimmune (e.g. multiple sclerosis, rheumatoid arthritis), and eye diseases (e.g. glaucoma) (84). Primary VD has an inherited component. Subjects often indicate that their parents, in particular their mothers, also suffered from cold hands and other symptoms. It typically manifests itself during puberty and declines with age. VD appears more frequently in women than men (112, 163). In females the symptoms often mitigate after menopause but can increase again when patients are treated with estrogen-replacement therapy (84).

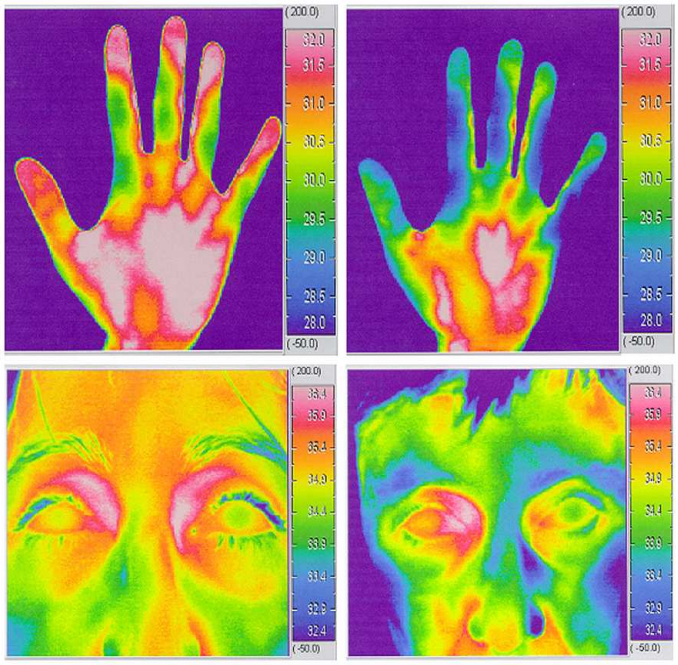


Figure 7. *Thermography. Comparison between a subject without vascular dysregulation (VD) (left) and with VD (right) displays a cold face and hand in VD and a warm picture of a subject without VD (Figure from (93)).*

The principal symptoms of subjects with primary VD are cold hands and feet (84) (Figure 7). Some patients suffer from migraine (84). Subjects with primary VD generally have low body mass index, but spasm can occur even in obese subjects (84). Some subjects suffer from low blood pressure (caused mainly by an increased loss of salt in the proximal tubuli of the kidney due to a mild stimulation of the endothelin B-receptors (157)) with, mostly nocturnal, "dips" or "overdips" whilst young but then exhibit a higher blood pressure as they get older (84). Additionally they show a reduced feeling of thirst (193) (this can be explained by a mild increase in Endothelin-1 having an anti-dipsogenic effect in the center of thirst (hypothalamus) via Prostaglandin-E_2 (82, 186)), and they more often suffer from migraine (88).The sensitivity for certain groups of drugs such as calcium channel blockers and systemic beta-blockers is increased in those subjects (84) probably due to differences in the expression pattern of multidrug resistance (MDR) proteins which are involved in the pharmacokinetic of drugs. Acute inhibition or decreased expression of MDR proteins may result in an enhanced uptake and systemic accumulation of drugs (217). They often have a meticulous personality and are successful in their professions (83). Additionally an epidemiological study (201) revealed that in women with VD (WVD) the vasospastic diathesis is not only associated with DIS but also with a higher level of anger/aggression than controls. They seem to accumulate their anger and not be able to relieve the tension evoked by this accumulation.

As mentioned above WVD have on an average a longer sleep-onset latency, especially when they are cold (159, 119). As warm feet are a prerequisite for a rapid sleep onset (117) the prolonged sleep-onset times in subjects with VD are explainable by their colder feet and therefore longer time to warm them up. In terms of circulation they respond more strongly with vasoconstriction to mechanical stress (e.g. whiplash injury), psychological stress, or cold (84). Why VD occurs more often in women than men (163) remains to be clarified. However, the fact that the symptom manifests in puberty and decreases with age indicates that hormones, in particular estrogens, play a role. This explains why the syndrome can aggravate when estrogen is substituted after menopause (83). Subjects with VD, although often presenting with cold hands, rarely have the classical symptoms of attacks with pale fingers, i.e. they do not have Raynaud‘s disease (84).

There are only scarce epidemiological data for VD. In a community-based survey of approximately 7000 people, almost 12 percent answered yes to the question "Are

either your fingertips or toes unusually sensitive to cold temperatures?" (131). A prevalence of VD of more than 30% of all age women has been reported for a Japanese population (152), and a survey in Switzerland revealed that 31% of women and 7% of men between the age of 20-40 years experience symptoms of VD (119). The prevalence range in the general population for Raynaud's disease is relatively high (between 5 and 10%, depending on methodology) (37, 183, 212). VD is mostly relatively harmless and does not need treatment. There is evidence that the syndrome does, however, predispose toward some diseases, such as normal-tension glaucoma, myocardial or cerebral ischemia (84).

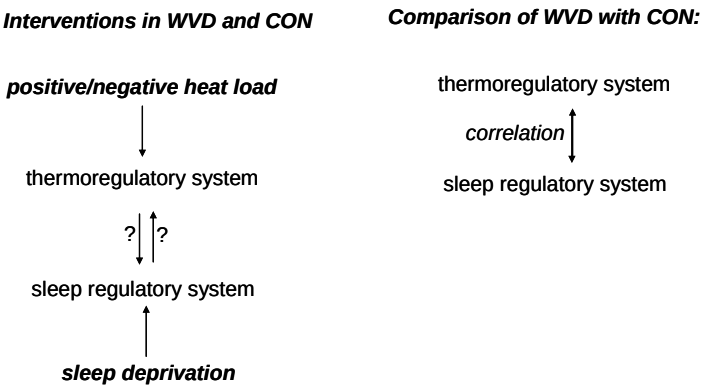
Whereas constriction in resistance vessels might be involved in the pathogenesis of systemic hypertension the dysregulation in VD is generally localized to the small arterioles and venules and the capillaries. Endothelin-1, a very potent vasoconstrictor protein, which is synthesized in the endothelium of blood vessels, seems to play a role in the genesis of VD (84).

There is no gold standard for the diagnosis of primary vascular dysregulation syndrome. The most specific and sensitive diagnostic tool for vasospasm is angiography but this diagnostic intervention is often too invasive. The most often used diagnostic test is cold provocation in nail fold capillaromicroscopy (172). The capillaries of the nail folds can be directly visualized under the microscope. A reduced baseline blood flow velocity and especially a prolonged flow stop after cold provocation, can be observed. The clinical diagnostic criterion for a VD is a blood standstill for ≥12sec after a cold CO_2 provocation (84, 86).

OBJECTIVES AND STRUCTURE OF THE THESIS

The general aim of the studies presented in this thesis is to extend our knowledge of thermoregulatory processes underlying sleep regulation.

The basis of the methodological approach is to describe the relationship between thermoregulation and sleep and their mutual influence by comparison of two populations in terms of their basal thermophysiological differences and sleep properties (correlative findings), and in terms of the response of these properties to specific stimuli (intervention studies). A group of women (WVD) with a diathesis of increased heat retention, i.e. with a vascular dysregulation, hence cold hands and feet and prolonged sleep onset latencies (SOL), will be compared to a group of women without such dispositions (CON).



Based on recent findings, thermoregulatory heat loss mechanisms appear to be relevant for ensuring an appropriate phase relationship between the circadian system and the sleep-wake cycle. Phase of entrainment largely determines normal, undisturbed sleep together with short SOL - an abnormal phase of entrainment could thus be a cause of sleep disturbances (as it is known for delayed sleep phase syndrome). Therefore subjects suffering from vascular dysregulation with concomitant difficulties initiating sleep should first be chronobiologically characterized to discover possible circadian maladjustments.

To assess homeostatic and circadian influences on sleep, wake, and the thermoregulatory system, a constant routine protocol (CR) was designed comprising a 40-h sleep deprivation (SD) preceded by a baseline (BL) and followed by a recovery (RE) night sleep. The CR provides information for a chronobiological

characterization of the subjects, i.e. defining the phases of CBT and melatonin rhythms and their relationship with the sleep-wake cycle (Chapter 2). SD provides a stimulus of enhanced homeostatic sleep pressure. The effects of such a stimulus on thermophysiological processes will be compared with the physiological values before in WVD and CON (Chapter 2). No studies exist of sleep-EEG characteristics (sleep stage and spectral analyses) in subject groups with different thermophysiological properties. But it is well known that temperature can influence sleep by CBT alterations in the evening (e.g. by passive or active body heat load), and can lead to homeostatic thermoregulatory after-effects, i.e. body heat loss via distal vasodilatation as well as reduced sleep onset latency and increased SWS. WVD exhibit different thermophysiological properties which may have an impact on their sleep pattern. The effect of SD on sleep (e.g. SOL, sleep architecture) in WVD and CON will be determined by performing sleep stage and EEG power spectral analyses on BL and RE (Chapter 3).

No controlled study exists showing temperature effects (e.g. by passive or active body heat load; positive heat load) on sleep in the afternoon, at a circadian phase of relative CBT stability (no surplus of body heat loss), and no controlled study has been carried out showing effects of a cold load (negative heat load) on sleep. The homeostatic adjustment after heat and cold loads resembles the thermoregulatory effects of the falling and rising limbs of the CBT in the evening and morning, respectively. In Chapter 4 this has been investigated by a temperature stimulus under controlled randomized cross-over design conditions. This study investigated whether sleep in CON is dependent on body heat loss (after warm bathing, CBT increase) or body heat gain (after cool bathing, CBT decrease (without shivering)) compared with a neutral bath (no change in CBT). The same protocol was also applied to WVD to compare their behavior regarding a temperature intervention prior to a sleep episode in comparison to CON. This should additionally give insight into the effects of heat load interventions in a "model" thermoregulatory disorder such as vascular dysregulation in terms of an improvement of the concomitant difficulties initiating sleep, with the additional aspect of therapeutic relevance.

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CHAPTER 2

CHRONOBIOLOGICAL CHARACTERIZATION OF WOMEN WITH PRIMARY VASOSPASTIC SYNDROME: BODY HEAT LOSS CAPACITY IN RELATION TO SLEEP INITIATION AND PHASE OF ENTRAINMENT

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ABSTRACT

Women with primary vasospastic syndrome (VS) but otherwise healthy exhibit a functional disorder of vascular regulation (main symptom: cold extremities), and often suffer from difficulties initiating sleep (DIS). Diverse studies have shown a close association between distal vasodilatation before lights off and a rapid onset of sleep. Therefore, we hypothesized that DIS in women with VS could be due to a reduced heat loss capacity in the evening i.e. subjects are physiologically not ready for sleep. The aim of the study was to elucidate whether women having both VS and DIS (WVD) or not (controls, CON) show different circadian characteristics (e.g. phase delay of the circadian thermoregulatory system with respect to the sleep-wake cycle).

Healthy young women (N=9 WVD and N=9 CON) completed a 40-h constant routine protocol (CR, adjusted to habitual bedtime), before and after a 8-h sleep episode. Skin temperatures (off-line calculated as distal-proximal skin temperature gradient, DPG), and core body temperature (CBT, rectal) were continuously recorded. Half-hourly saliva samples were collected for melatonin assay and subjective sleepiness was assessed on the Karolinska Sleepiness Scale (KSS).

In comparison to CON, WVD showed no differences in habitual bed times, but a 1 h circadian phase delay of dim-light-melatonin-onset (h after lights on: WVD 14.6 ± 0.3h; CON 13.5 ± 0.2h; p=0.01). Similar phase shifts were observed in CBT, DPG, and KSS ratings. In conclusion, WVD exhibit a phase delay of the endogenous circadian system with respect to their habitual sleep-wake cycle, which could be a cause of DIS.

INTRODUCTION

In humans and other mammals, circadian rhythms are generated by a self-sustaining circadian pacemaker located in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus (30). The SCN drives circadian rhythms in physiological processes, which are synchronized to the outside world mainly by the solar light-dark cycle. Core body temperature (CBT) has been investigated as a robust and convenient circadian marker rhythm in humans (62, 65, 4). However, the circadian rhythm of CBT is more than a marker (17, 61) – thermoregulatory changes are intimately coupled to sleepiness and sleep induction.

The homeostatic control of CBT is mediated by a hierarchically organized set of neuronal mechanisms, with the preoptic anterior hypothalamus (POAH) at the top of the hierarchy (57). CBT is regulated within narrow limits around 37℃ by a complex feedback system (55). In order to keep CBT within these limits POAH can activate heat loss (e.g. distal vasodilatation, sweating) and heat gain mechanisms (e.g. distal vasoconstriction, metabolic heat production). In addition to the homeostatic regulation, a rostral projection from the SCN to the preoptic areas provides circadian modulation of CBT (48). The circadian rhythm of CBT is determined by both changes in heat production and heat loss. CBT declines when heat loss surpasses heat production in the evening, and vice versa in the morning (6, 40). There is growing evidence that body heat loss in the evening via increased distal skin temperatures is the crucial thermoregulatory function for induction of sleepiness and sleep (26, 37). The best indirect marker of this readiness for sleep may be the distal-proximal skin temperature gradient (DPG), a measure which has been externally validated to distal skin blood flow (56). The rise of DPG about 90 min before lights off is a good predictor for a rapid onset of sleep (37). Because the circadian regulation of CBT is intimately coupled with the circadian regulation of sleepiness and sleep induction (12), the phase relationships (‘phase of entrainment’) between endogenous circadian rhythmicity of the thermoregulatory system and the sleep-wake cycle are important for good sleep. Disruption of phase of entrainment can profoundly influence human health, being linked e.g. to mood disorders, jetlag, coronary heart disease and sleep disorders, such as difficulties initiating sleep (DIS) (22, 42, 45). Therefore studying DIS in relation to the phase relationship between endogenous circadian rhythmicity of

CBT regulation and the sleep-wake cycle may reveal clues to underlying mechanisms.

In a first of a series of studies, we have chosen a strategy to show this relationship in two selected extreme groups of subjects, one with impaired heat loss capacity (i.e. vasospastic syndrome, VS) in addition to DIS, comparing with a group of controls having not these problems. This extreme group comparison has the advantage allowing a study with relative small sample sizes in both groups, however, with the limitation that no conclusion can be drawn, whether one of the two symptoms (VS or DIS) alone could also produce similar results. Nevertheless, conclusive interpretation can be drawn with respect to subjects showing the combination of VS and DIS. Subjects with VS represent a part of the general population (mostly women before menopause) with a diathesis of responding with spasm, in particular in the distal extremities (hands, feet) to stimuli like cold or emotional stress (24). The VS is a similar but weaker form of Raynaud's disease (the classical symptom of Raynaud's phenomenon - the triphasic color changes of the digits of the hands and feet from white to blue to red - is not necessary for its definition). In a recent epidemiological study carried out in a representative urban Swiss population (age: 20-40 years) it could be shown that about 30% of women exhibit a VS, in contrast to only 7% of men and that the relative risk of DIS in these subjects is doubled (33). This large survey confirmed our previous findings in a small sample of women with VS and normal tension glaucoma who exhibited significantly prolonged sleep onset latency (SOL) in comparison to controls (49, 51). Therefore prolonged SOL in these subjects could be associated with their impaired capacity for distal vasodilatation and heat loss before habitual bedtime (33). Based on the survey findings we focused on women.

The purpose of the present study was the chronobiological characterization of women with VS and DIS (WVD) compared with control women (CON), using a "constant routine protocol" (CR) that controls for the main masking effects such as locomotor activity, large meals, changes in light condition and posture on the endogenous timing system (15, 20, 40, 46). To clarify the relationship between DIS and VS, three a priori hypotheses were tested, all of which could lead to a higher vasoconstriction level (lower DPG values) before their habitual bedtimes and hence leading to a longer SOL:

1) A circadian phase delay of the thermoregulatory system, leading to a changed phase of entrainment in comparison to habitual bedtimes.
2) A larger circadian amplitude of distal skin temperature (expressed by DPG).
3) A normal circadian rhythm of the thermoregulatory system with respect to phase and amplitude but on a generally lower 24-h mean level (e.g. of DPG).

All these possibilities could lead to lower DPG levels before habitual bedtimes i.e. subjects with VS are simply physiologically not ready for sleep.

To test these hypotheses, the circadian time course of CBT, distal and proximal skin temperatures, and subjective ratings of sleepiness were compared with an established circadian reference rhythm, that of melatonin production, measured during a CR, adjusted to their habitual bedtimes. Melatonin production itself is known to be related to circadian thermoregulation and sleepiness (for review (36, 60)), providing therefore additional information about mechanisms of phase of entrainment.

METHODS

Subjects

Two groups of healthy young women (WVD N = 9, and CON N = 9) were recruited via poster advertisements at the University of Basel and via announcements on the internet (for their physiological characteristics see Table 1). Based on the homogenous selection of the two study groups (CON vs. WVD) and on our very stringent controlled CR protocol, the main target variables (SOL, DPG, CBT) can be statistically analyzed with N=9 for each group to have a statistical power of at least 80% e.g. a relevant between-group difference in DPG of 1°C with expected standard errors of the mean (SEM) of 0.25°C will lead to a power of 80% (e.g. t-test); a relevant between-group difference in sleep onset latency to sleep stage 2 (SOL) of 10 min with expected SEM of 2 min will lead to a power of 86% (e.g. t-test); similar criteria are valid for all the other variables. Intraindividual changes can be detected even with a higher power, or in other words, smaller standardized differences will be significantly detected (SOL, SEM: 2 min, Δ 5min, power: at least 0.8; DPG, SEM: 0.2°C, Δ 0.5°C, power: at least 0.8). All subjects had to successfully complete the following screening questionnaires: The Torsvall-Åkerstedt morning-evening-type questionnaire (59) and two questionnaires covering sleep habits, sleep quality, life habits, physical health, medical history and thermophysiological behavior. Exclusion criteria were extreme morning or evening types (M/E-types) (ratings ≤14 and ≥21

points), chronic or current major medical illness or injury, amenorrhea or irregular menstrual cycles, smoking, intake of over-the-counter or prescription medications (including oral contraceptives or other hormonal treatments) or illicit substances, shift work within 3 months or transmeridian travel within 1 month prior to the study, excessive caffeine (i.e. > 300mg) and alcohol consumption (i.e. > 1 beverage per day).

Subjects who fulfilled the described criteria were subjected to a finger nailfold video capillary microscopy to objectively document their self-ratings about cold or warm extremities, respectively (inclusion criteria: blood standstill for ≥ 12 sec = WVD, < 12 sec = CON) (23, 27). Additionally, the nailfold skin temperature was measured. After a physical examination to exclude any medical disorders, a polysomnographically recorded screening night in the laboratory was performed to test their ability to sleep in a new environment, to exclude primary sleep disorders (i.e. insomnia) and to assess the sleep onset latency to sleep stage 2 (≥ 20 min for WVD, < 15 min for CON, see Table 1).

All selected subjects entered the study between the fourteenth and the first day of their menstrual cycle in order to complete the experiment within the luteal phase. During 7 days before their admission to the laboratory (baseline week) subjects were instructed to maintain a regular sleep-wake schedule (bedtimes and wake times within ± 60 min of self-selected target time scheduled 8 h apart). Adherence to this regular schedule was verified with a wrist activity monitor (Cambridge Neurotechnologies®, UK) and sleep-wake logs. They were also instructed to abstain from excessive caffeine and alcohol consumption (definition see above) as well as heavy physical exercise. The nature, purpose, and risks of the study were explained before subjects gave their written consent. It was explicitly permitted to stop the experiment at any time. The study protocol, screening questionnaires and consent form were approved by the local ethical committee (‘Ethikkomission beider Basel’) for research on human subjects and conformed to the Declaration of Helsinki. All 18 subjects completed the study without any complaints.

Study design and protocol

After the baseline week, subjects reported to the laboratory 2 h before their habitual bedtime for an adaptation night (the timing of their sleep–wake schedule was calculated in such way that the sleep episode was centered at the midpoint of each subject's habitual sleep episode as assessed by actigraphy during the baseline week). They were prepared for continuous polysomnographic and temperature recording. Subjects were allocated to a sound attenuated, air-conditioned chronobiology room controlled for light (< 8 lux [typically 3-5 lux at the angle of gaze] during wakefulness and 0 lux during scheduled sleep), ambient air temperature (22℃) and relative humidity (55%). The following 8 h of wakefulness on Day 1 (D1) were used to adjust the subjects to the experimental dim light conditions (< 8 lux). They were allowed to walk around the laboratory. To assure no light input stronger than 8 lux when they walked out of the dimmed room, they had to wear sunglasses. In the afternoon of D1, after having self-inserted the rectal probe, subjects laid down exactly 30 minutes before the start of the protocol (8 h before lights off), and the remaining thermocouples were immediately attached. Subjects were covered with a blanket but could adjust their bedcovers to maintain thermal comfort. Isocaloric snacks were given hourly and water was available ad libitum. After a second 8 h sleep episode (Night 1 [N1]), the subjects followed a 40 h CR (Day 2 and 3 [D2, D3]) with constant wakefulness. After a third 8 h sleep episode (recovery night, Night 3 [N3]), the protocol was continued for a further 1.5 h on the morning of Day 4 (D4).

Physiological measurements

Salivary melatonin

Saliva collections (1-2 ml) were scheduled every 30 min during wakefulness. The samples were immediately refrigerated at 5℃, centr ifuged within 2 days and stored at -20℃. 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 (64)).

Subjective ratings of sleepiness

The 9-point Karolinska Sleepiness Scale (KSS) was used to assess subjective sleepiness at half-hourly intervals (1).

Thermometry

Temperature data were continuously recorded by a computerized system (System Hofstetter, SHS Allschwil, Switzerland) in 20-sec intervals and collapsed off-line into 15-min intervals. Rectal temperature as a measure of CBT was registered by a thermocouple (polyoxymethylene probe: 2-mm diameter, copper-constantan, accuracy: 0.01℃; Interstar, Cham, Switzerland; Therm, type 5500–3, Ahlborn, Holzkirchen, Germany) inserted 10 cm into the rectum and maintained in place by surgical tape. Skin temperatures were also registered by thermocouples (silver disk: 1-cm diameter, copper-constantan, model P 224, Prof. Schwamm, Ahlborn; accuracy: 60.01℃; Therm, type 5500–3, Ahlborn) fixed to the skin with thin air-permeable adhesive surgical tape (Fixomull, Beiersdorf, Hamburg, Germany). The body temperatures were measured on 9 body sites: rectal (T_{re}), midforehead (T_{fh}), 1cm above the navel (T_{st}), right infraclavicular area (T_{ic}), center of back of hands (T_{ha}), middle of foot insteps (T_{fo}), and midthigh on musculus rectus femoris (T_{th}). Raw data of temperatures were inspected visually, and data segments that were affected, e.g. by probe slips of malfunctioning of the temperature sensors, were removed. These missing data were replaced by value derived from a linear interpolation procedure. To reduce short-term fluctuations and the number of time segments, data were averaged into 15-min bins. For theoretical reasons (5) and because of similarities to our earlier study (40), we combined T_{ha} and T_{fo} to provide an average for the distal skin temperature, and T_{fh}, T_{st}, T_{ic}, T_{th} for the average proximal skin temperature (T_{prox}). A weighted average was calculated for T_{prox} according to Ref. (29) with slight modifications: forehead x 0.093, thigh x 0.347, infraclavicular region x 0.266, and stomach x 0.294.

Sleep onset latency

Sleep was polysomnographically recorded by a digital recording system using the VITAPORT digital ambulatory sleep recorder (Vitaport-3 digital recorder, TEMEC® Instruments BV, Kerkrade, The Netherlands) and sleep stages were visually scored on a 20-sec basis according to standard criteria (for details see (32)). The sleep

analysis will be published elsewhere. SOL was defined as the time interval between lights off and the occurrence of the first 20-sec sleep epoch of sleep stage 2.

Data Analysis

Analysis of the dynamics before, during and after the CR

Analyses of the time course of D1 and N1 deliver details how the thermoregulatory system of WVD in comparison to CON differs with respect to sleep induction and sleep. The time course of the thermoregulatory variables over the time span from 2 h after lying down on D1 until end of N1 (13 h) was analyzed by a two-way analysis of variance for repeated measures (rANOVA) with the factor *time* (13 x 1-h bins) and factor *group* (WVD vs. CON).

The CR protocol reduces on the one hand the most important masking effects (e.g. food intake, activity, postural changes) but on the other hand also induces other masking effects (e.g. sleepiness, long term changes of constant bed rest). In order to reveal possible influences of the CR protocol on the thermoregulatory system, melatonin and sleepiness, a two-way rANOVA was performed with factor *day* (D2 vs. D3) and factor *group* (WVD vs. CON), each level comprising an 8-h episode between 3 h and 11 h after habitual lights on. The selected timing of the 8-h episodes allows a comparison between D2 and D3 without possible influences of circadian phase shifts.

Analyses of the time course of N3 and D4 deliver details how the thermoregulatory system of WVD differs to CON after a 40-h sleep deprivation with respect to recovery sleep and the 1-h episode afterwards. The time course of the thermoregulatory variables comprising the time span between lights off on D3 until 1h after lights on on D4 (9 h), was analyzed by a two-way rANOVA with the factor *time* (9 x 1-h bins) and factor *group* (WVD vs. CON). Melatonin and sleepiness were not measured during sleep.

Analysis of phase markers

To ensure that circadian measurements were made under basal conditions, the first 5 h of constant routine data on D2 were excluded from analysis to eliminate any residual effects of sleep on the tested variables (10). In order to reduce effects of sleep preparations on the tested variables the last 2 h of data on D3 were also

omitted. Therefore data of 33-h CR were analyzed. To determine circadian characteristics we focused on melatonin production, which provides accurate information about the endogenous circadian rhythm (9).

Dim light melatonin onset time (DLMO) (43), as determined by linear interpolation of the evening melatonin rise across a 3 pg/ml threshold, was taken to estimate the phase of melatonin production. For analysis, all of the 30 min samples were used. Maximum values were extracted in order to get information about circadian amplitude of salivary melatonin concentration. As an accurate method to determine phase, amplitude and mesor of the melatonin rhythm, non-orthogonal spectral analysis was used to fit a three harmonic model without correlated noise to the data (10, 16, 41). The fitted maximum of the salivary melatonin rhythm was used as a marker of the phase of the endogenous circadian pacemaker. The period of the fundamental component of the model was constrained between 23 and 25 h. For CBT rhythm analysis a two harmonic model with correlated noise was used (10, 16).

The phase relationship between WVD and CON regarding CBT, melatonin, DPG, and subjective ratings of sleepiness (KSS), was calculated using cross-correlation analysis. These analyses were performed using the circadian time course during the CR of a 33-h episode starting 5 h after lights on on D2 and ending 2 h before lights off on D3. To purify original sleepiness and temperature data from additional long-term trends due to the CR, residuals to a linear regression line were taken for the cross-correlation analysis. Cross-correlations were calculated for time lags of $\pm$ 480 min. Time lags (Δmin) of maximum or minimum *r*-values were extracted from individual cross-correlation-curves (for details see (36)).

Statistical analyses

The statistical packages StatView™ 5.0 and SuperANOVA™ (Abacus Concepts, Berkeley, California, USA), and STATISTICA 6 ™ for Windows (StatSoft Inc., Tulsa, USA) were used.

Analyses of time courses were performed by cross-correlation analyses and by two -way rANOVA with grouping factor *group* (WVD vs. CON) and repeated factor *time* (or *day*). All *P* values derived from rANOVAs were based on Huyhn-Feldt corrected degrees of freedom, but the original degrees of freedom are reported. For *post-hoc*

comparisons Fisher's PLSD with alpha-correction for multiple comparisons according to Curran-Everett (14) were calculated. For statistical analyses between WVD and CON without an a priori hypothesis, the threshold for alpha-errors was set at $P < 0.05$ (two-sided, not especially indicated), otherwise at $P < 0.1$ (one-sided, indicated by †). The Mann-Whitney U-test was used to reveal significant differences between WVD and CON. Means ± SEM values are presented.

RESULTS

Characteristics of subjects

Table 1 presents the descriptive and inferential statistics for age, BMI, finger temperature, and data from the sleep/wake diary (including actimetry), sleep questionnaires and polysomnographic recordings of sleep onset latency to sleep stage 2. WVD and CON do not significantly differ in age and BMI, whereas the measured finger temperatures of WVD are significantly lower. Neither habitual time of lights off and lights on nor M/E-type differs significantly, indicating no differences between the two groups in their sleep-wake cycle. The subjective rating of difficulties initiating sleep in WVD was polysomnographically confirmed by significant longer sleep onset latencies not only in the screening night and N1 but also in N3 even after 40-h sleep deprivation.

Table 1. Physiological characterization of the study participants

Variable	CON	WVD	*P* value (U- test) CON vs. WVD
Age, years	25.1 ± 1.7	24.2 ± 1.2	0.50
BMI, kg/m^2	20.85 ± 0.6	20.82 ± 0.54	0.96
Morning/Evening-type (59)	16.7 ± 0.9	16.0 ± 1.6	0.86
Fingertemp, ℃	32.83 ± 0.49	28.5 ± 0.99	**0.002** †
habitual lights off time, clock time	23:46 ± 0:07	23:25 ± 0:12	0.17
habitual lights on time, clock time	07:44 ± 0:07	07:24 ± 0:12	0.11
SOL baseline week, subjective ratings, min	15.02 ± 3.27	31.59 ± 4.46	**0.0025** †
SOL screening night, polysomnography, min	10.04 ± 1.14	37.41 ± 10.47	**0.0001** †
SOL N1, polysomnography, min	8.82 ± 1.24	19.11 ± 3.54	**0.01** †
SOL N3, polysomnography, min	5.37 ± 1.13	9.78 ± 1.48	**0.015** †

Values are means ± SEM. Polysomnographically obtained sleep onset latency (SOL) refers to the interval between lights off and the first epoch of sleep stage 2. SOL, sleep onset latency to sleep stage 2. †, one-sided.

Analysis of Salivary Melatonin, CBT, Skin Temperatures, and Sleepiness

Analysis of the time course of the first 5 h CR on D1 provides information about the transition phase from daily life to the controlled CR condition. Analysis of the time course of the succeeding night (N1) delivers details as to how the thermoregulatory system of WVD differs in comparison to CON with respect to sleep induction and sleep. For the CR, systematic changes from D2 to D3 (e.g. caused by long bed rest and sleep deprivation) were tested. Additionally, the influence of recovery sleep on the variables was tested during N3 and 1 h afterwards.

WVD are compared to CON also with respect to circadian amplitude and phase of melatonin and CBT. The time span between 5 h after lights on on D2 and 2 h before lights off on D3 was analyzed (33 h). DLMO, and sinusoid-based analyses of melatonin and CBT were performed to determine circadian phase and cross-correlation analyses were used to define phase relationships between the variables (phase of entrainment).

Salivary Melatonin

Half-hourly mean values of salivary melatonin concentration with typical high levels during the night are shown in Figure 1.

Extracted DLMO time of WVD occurs significantly later than in CON (pooled DLMO [D1, D2 and D3], hours after lights on: 14.63 ± 0.30 h and 13.54 ± 0.23 h, main effect *group*: $F_{1,16}$ = 8.31; P = 0.01). WVD and CON do not significantly differ with respect to the experimental days (main effect *day*: $F_{2,16}$ = 0.89; P = 0.42), nor reveal any interaction term significance (*day x group*: $F_{1,32}$ = 0.04; P = 0.96). This analysis indicates that WVD in comparison to CON exhibit a phase-delayed circadian rhythm in salivary melatonin concentrations.

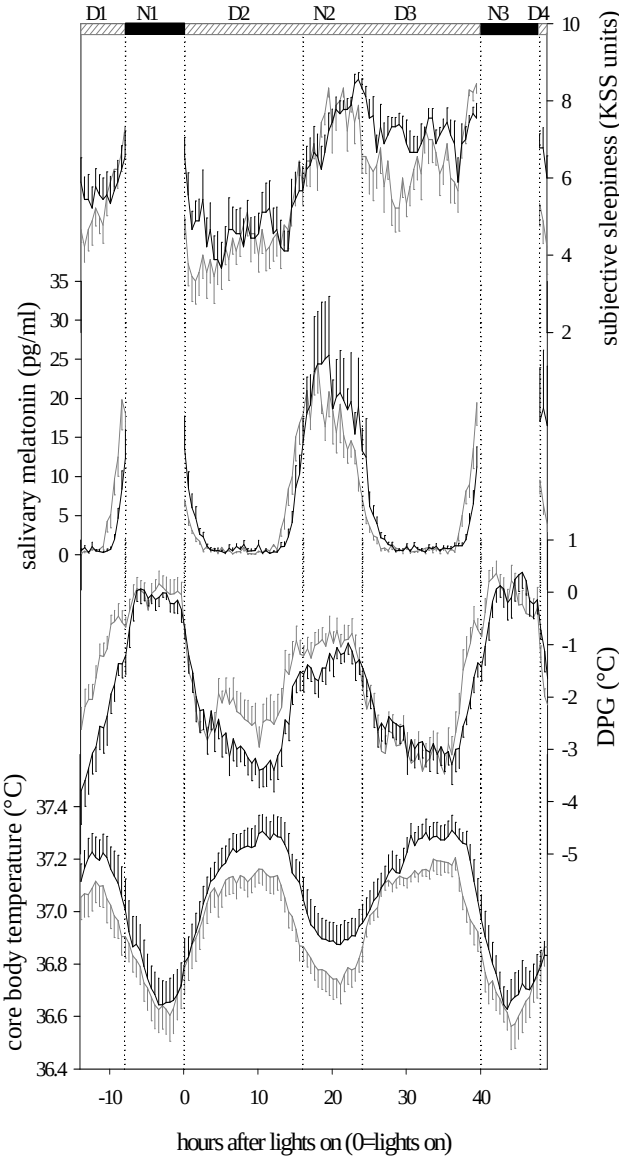

Figure 1. *Subjective sleepiness, Melatonin, and core body temperature waveforms averaged with respect to usual wake time for CON and WVD. Black, WVD (N=9); gray, CON (N=9). Black bar on the top, time of scheduled sleep episode. Data were plotted with respect to scheduled wake time, with scheduled wake time assigned a value of 0 h. Temperature data were first averaged in 15 min bins for each subject; data for all subjects in each group were then averaged, and mean is shown with + or – SEM. DPG, distal-proximal skin temperature gradient. KSS, Karolinska Sleepiness Scale.*

Table 2. Effect of sleep on thermoregulatory variables, subjective ratings of sleepiness (KSS) and salivary melatonin of CON and WVD during D1 and N1

Variable	*Group*		*Time*		*Time x Group*	
CBT	*F* (1,16) = 0.35	*P* = 0.56	*F* (12,192) = 81.15	*P* < **0.0001**	*F* (12,192) = 1.12	*P* = 0.35
DPG	*F* (1,16) = 3.98	*P* = **0.03** †	*F* (12,192) = 50.63	*P* < **0.0001**	*F* (12,192) = 3.32	*P* = **0.0001** †
KSS	*F* (1,16) = 0.30	*P* = 0.3 †	*F* (6,96) = 4.53	*P* = **0.0002**	*F* (6,96) = 1.39	*P* = 0.115 †
Melatonin	*F* (1,16) = 6.95	*P* < **0.02**	*F* (6,96) = 51.07	*P* < **0.0001**	*F* (6,96) = 6.28	*P* < **0.0001**

Two-way rANOVAs with factors group (CON vs. WVD), and time (for the thermoregulatory variables: 5 h before lights off until 8 h after lights off [total 13 x 1 h-bins]; for melatonin and KSS: 7 h before lights off until lights off [total 7 x 1 h-bins]). Melatonin and KSS were measured only during the wake phase; therefore, df of factor time is 6 for these variables. CBT, core body temperature. DPG, distal-proximal skin temperature gradient. KSS, Karolinska Sleepiness Scale. †, one-sided.

Two-way rANOVA for D1 reveals a significant interaction term *time x group* (Table 2) which also can be interpreted as a later onset of melatonin production in WVD than in CON (see above). This interpretation could be confirmed in further analyses (see

below). Additionally, significant main effects *time* and *group* are found emphasizing the strong influence of the circadian modulation of melatonin production and the phase shift between WVD and CON. Neither the CR protocol (including 24-h wakefulness and sustained supine posture; D1 vs. D2; Table 3) nor the first hour after recovery sleep (N3) reveals significant differences between WVD and CON (Table 4).

Table 3. Effect of 24-h sleep deprivation on thermoregulatory variables, subjective ratings of sleepiness (KSS) and salivary melatonin in CON and WVD: Comparison of a 8 h episode at the same circadian phase on D2 and D3 of the CR

Variable	*Group*		*Day*		*Day x Group*	
CBT	F (1,16) = 2.13	P = 0.16	F (1,16) = 1.02	P = 0.33	F (1,16) = 0.16	P = 0.70
DPG	F (1,16) = 0.08	P = 0.78	F (1,16) = 1.98	P = 0.18	F (1,16) = 6.31	P = **0.02**
KSS	F (1,16) = 1.50	P = 0.24	F (1,16) = 78.79	$P <$ **0.0001**	F (1,16) = 2.04	P = 0.17
Melatonin	F (1,16) = 0.36	P = 0.56	F (1,16) = 2.90	P = 0.11	F (1,16) = 0.04	P = 0.84

Two-way rANOVAs with factors group (CON vs. WVD), and day (8 h of D2 and D3 for thermoregulatory variables, KSS, and melatonin [3 h after time of lights on until 5 h before lights off]. CBT, core body temperature. DPG, distal-proximal skin temperature gradient. KSS, Karolinska Sleepiness Scale.

Table 4. Effect of sleep on thermoregulatory variables, subjective ratings of sleepiness (KSS) and salivary melatonin of CON and WVD during N3 and the following morning (D4)

Variable	*Group*		*Time*		*Time x Group*	
CBT	F (1,16) = 0.32	P = 0.58	F (8,128) = 8.22	$P <$ **0.0001**	F (8,128) = 0.81	P = 0.471
DPG	F (1,16) = 0.09	P = 0.77	F (8,128) = 11.16	$P <$ **0.0001**	F (8,128) = 2.52	P = **0.036**
KSS	F (1,16) = 4.73	P = **0.045**	F (3,48) = 5.72	P = **0.0073**	F (3,48) = 0.195	P = 0.827
Melatonin	F (1,16) = 2.25	P = 0.145	F (3,48) = 7.82	$P <$ **0.0068**	F (3.98) = 1.97	P = 0.176

Two-way rANOVAs with factors group (CON vs. WVD), and time (for the thermoregulatory variables: 8 h after lights off and 1 h after lights on [total 9 x 1-h bins]; melatonin and KSS was measured only during the wake phase: 1.5 h after lights on [total 4 values]). CBT, core body temperature. DPG, distal-proximal skin temperature gradient. KSS, Karolinska Sleepiness Scale.

A three-harmonic non-orthogonal spectral model without correlated noise was used to estimate the circadian phase, amplitude and 24-h mean level. This model reveals a significant phase delay for the fitted maximum of the melatonin rhythm of WVD compared to CON (19.19 ± 0.37 h and 18.02 ± 0.36 h after lights on, $P < 0.01$). No significant differences regarding fitted amplitude (WVD: 13.0 ± 3.3 pg/ml vs. CON: 11.9 ± 1.4 pg/ml; $P = 0.57$) and 24-h mean level (WVD: 8.8 ± 2.0 pg/ml vs. CON: 7.7 ± 0.8 pg/ml; $P = 0.76$) were found.

To provide additional information regarding the phase relationship between CON and WVD, cross-correlation analyses were performed with averaged melatonin values (CON, N = 9 subjects) as the reference rhythm (Table 5). A significant phase delay was found between WVD and CON (-51.66 ± 12.53 min *P* = 0.003; Table 5).

Core body temperature (CBT)

Typical time courses of CBT (15-min bins) for WVD and CON are shown in Figure 1. The time course of CBT on D1 and N1 do not statistically differ between WVD and CON (interaction term *time x group*, n.s., and main effect *group,* n.s., Table 2). The strong influence of the nightly drop in CBT leads to a significant main effect *time* (Table 2).

The effect of 24-h wakefulness and sustained supine posture in a CR protocol during a 8-h time segment on D2 and D3 at a similar circadian phase was tested by a two-way rANOVA. Mean values of 8-h episodes at a circadian phase which is relatively unaffected by circadian phase shift effects were taken for rANOVA (see Methods). No significant interaction term *day x group* and no significant main effects (*day* and *group*; both n.s.) were found (Table 3). The time course during N3 and the first hour afterwards does not statistically differ between WVD and CON (Table 4).

Possible phase shifts between WVD and CON were calculated using a dual-harmonic non-orthogonal spectral model with correlated noise. It yielded a tendency to a difference between WVD and CON for the fundamental minimum and maximum of circadian CBT rhythm (21.86 ± 0.40 h vs. 20.78 ± 0.37 h, *P* = 0.07, after lights on, and 9.73 ± 0.41 h vs. 8.57 ± 0.38 h, after lights on, *P* = 0.06). That is, the fundamental minimum of WVD tends to occur later, indicating a phase delay in the circadian CBT rhythm. 24-h mean value tends to remain at a higher level in WVD than CON (37.10 ± 0.06℃ vs. 36.99 ± 0.05℃, *P* = 0.06). The fitted amplitude of CBT over the CR shows no difference between WVD and CON (0.24 ± 0.02℃ vs. 0.24 ± 0.02℃, *P* = 0.51).

Cross-correlation analyses exhibit a significant phase delay of CBT rhythm in WVD compared with CON (-60.00 ± 21.36 min, *P* = 0.02; Table 5).

Table 5. Phase relationship between CON and WVD

Variable	CON	WVD	*P* (U-test)
Melatonin	5.00 ± 10.31	-51.66 ± 12.53	*P* = **0.003** †
CBT	0.000 ± 12.99	-60.00 ± 21.36	*P* = **0.02** †
DPG	-10.01 ± 8.30	-50.00 ± 15.00	*P* = **0.0235** †
KSS	-20.00 ± 16.60	-79.17 ± 36.77	*P* = **0.025** †

Values are means ± SEM in min ± min. Maximum and minimum lags were extracted from individual cross-correlation curves. The mean time series of CON were taken as the reference time series (lag = 0). Individual time series of CON and WVD were cross-correlated to the mean time series of CON. +lag values, phase advances; -lag values, phase delays. CBT, core body temperature. DPG, distal-proximal skin temperature gradient. KSS, Karolinska Sleepiness Scale. CON values did not statistically differ from 0 (one-sample sign test). †, one-sided.

Distal-proximal skin temperature gradient (DPG)

The time course of DPG (15-min bins) for WVD and CON are shown in Figure 1. Table 2 summarizes the results of the two-way rANOVA of D1 and N1. A significant interaction term *time x group* was found. Inspection of the differences between WVD and CON reveals significant lower DPG values in the evening before lights off (WVD vs. CON mean 5 h segments; -2.65 ± 0.39°C vs. -1.46 ± 0.18°C, *P* = 0.015).

The effect of 24-h wakefulness and sustained supine posture in a CR protocol during a 8-h time segment on D2 and D3 at a similar circadian phase was tested by a two-way rANOVA. A significant interaction term *day x group* was found (Table 3). In comparison to CON, WVD reveal significant differences only on D2 but not on D3. The analysis of the time course during N3 and afterwards shows one hour after lights on a significant higher level in WVD than CON (significant interaction term: *Time x group*: *P* = 0.036; Table 4).

Subjective Sleepiness (KSS)

Subjective sleepiness on D1 shows no difference between WVD and CON (no significant main effect *group*, and interaction term *group x time,* Table 2). Based on the strong circadian effect on sleepiness in both groups in the evening, a significant main effect *time* was found (Table 2). A two-way rANOVA for the 8-h time segments on D2 and D3 reveals in both groups a similar significant increase in sleepiness during the CR protocol (significant main effect *day*, Table 2). After N3 KSS values are significantly higher in WVD than CON (significant main effect *group*, Table 4)

Comparison of circadian phase relationships between the variables

To compare circadian phase relationships between the variables (CBT, DPG and KSS) in relation to melatonin, cross-correlation analyses were performed with residuals after linear detrending (see METHODS; Table 6). Subjective ratings of sleepiness show a significant increase during the CR (two-way rANOVA on KSS: main factor *time*, $F(32,512) = 158.98$, $P < 0.0001$) reflecting the effect of sleep deprivation on sleepiness. No difference was found between WVD and CON regarding the phase relationships between DPG, KSS, CBT and the reference melatonin rhythm.

The phase relationships between CBT (pooled WVD and CON) and melatonin rhythm reveal a significant phase delay of CBT (-71.3 ± 14.6 min, $P < 0.0001$; Table 6). Similarly, a significant phase delay was found between KSS and melatonin (-110.0 ± 25.8 min, $P = 0.01$; Table 6). In contrast, no phase differences between DPG and melatonin curves were found, indicating phase-locked (inverse) patterns.

Table 6. Phase relationship between the variables

Variable	CON Lag to MEL (min)	WVD Lag to MEL (min)	*P* (U-test) CON vs. WVD	Pooled CON and WVD Lag to MEL (min)	*P* (sign-test) vs. 0 lag
CBT	-68.3 ± 16.1	-74.2 ± 25.5	*P* = 0.790	-71.3 ± 14.6	*P* < **0.0001**
DPG	-14.2 ± 19.8	-8.34 ± 28.6	*P* = 0.534	-11.3 ± 16.9	*P* = 0.45
KSS	108.3 ± 35.1	-111.7 ± 40.0	*P* = 0.965	-110.0 ± 25.8	*P* = **0.01**

Values are means ± SEM in min ± min. Maximum and minimum lags were extracted from individual cross-correlation curves. For each variable the mean time series of CON and WVD were taken as reference time series, respectively (lag=0). Individual time series of CON and WVD were cross-correlated to the mean time series of CON and WVD, respectively. + lag values, phase advances; - lag values, phase delays. CBT, core body temperature. DPG, distal-proximal skin temperature gradient. KSS, Karolinska Sleepiness Scale. MEL, melatonin.

Taken together, WVD show in comparison to CON a similar circadian phase shift in all variables of about 1 h, whereby the internal phase relationships between them remain constant within the groups. Circadian amplitudes (melatonin and CBT) are not different between WVD and CON. The homeostatic aspect of sleepiness regulation is also similar in WVD and CON. DPG, a measure of distal vasoconstriction and vasospasms, is significantly lower in WVD than CON, at least at the beginning of the CR on D1. During N1 the DPG does not differ between WVD and CON, however, vasoconstriction in WVD reappears during the next day (D2). On D3 and the following night (N3) no differences between WVD and CON are found. The short time segment after the recovery night N3 shows higher DPG and KSS values in WVD than CON.

DISCUSSION

The discussion section has been structured with respect to the three hypotheses formulated concerning differences in circadian phase, amplitude and 24-h mean level. The main finding of our study is that women with vasospastic syndrome and difficulties initiating sleep (WVD) exhibit in comparison to controls (CON) a significant phase delay of the circadian system by ca. 1 h. This finding favors the hypothesis that the circadian physiology in WVD does not sufficiently prepare the body for sleep initiation. This could lead to prolonged sleep onset latency found not only in the night directly before the CR, but also after 40-h sleep deprivation in the recovery night, when sleep pressure is high (see below). The phase delay of the circadian system could be measured by diverse variables to a similar extent, i.e. salivary melatonin concentration, CBT, DPG and subjective ratings of sleepiness (see Table 5). Therefore, a difference in the phase angle between circadian and sleep-wake rhythmicity of WVD (different internal 'phase of entrainment' (54)) could be the cause of DIS in this syndrome (see below).

It is well known that misalignment between the endogenous circadian system and the sleep-wake cycle (difference in phase of entrainment) can lead to sleep disturbances (including DIS), e.g. delayed or advanced sleep phase syndrome (8), shift work sleep disorder (19), jetlag syndrome (63), the non-24 h sleep/wake disorder (58) and extreme M/E-type (7, 21, 47). A condition of marked discrepancy in sleep timing between work and free days is found particularly in E-types (designated "social jetlag"). This leads to a considerable sleep debt on work days, for which they compensate on free days (66). Our large epidemiological survey was able to show that women with VS exhibit not only a prolonged SOL, but also a significant predisposition to E-types and social jetlag (38). All these disturbances are characterized by differences in internal and external phase of entrainment.
In contrast, we could show that WVD exhibit a selective difference in internal phase of entrainment with no differences in sleep timing (e.g. lights off time) compared with controls. This could indicate that a difference in internal phase of entrainment is crucial for DIS. Furthermore, a difference in internal phase of entrainment includes also changes in the thermoregulatory system relative to the sleep-wake cycle.

Earlier studies have shown that an increase in distal vasodilatation, and hence body heat loss (e.g. induced by exogenous melatonin, mild skin warming etc.), induces sleepiness and sleep initiation (26, 34, 36, 53), thereby changing internal phase of entrainment between the thermoregulatory system and the sleep-wake cycle. Thus, a different internal phase of entrainment, as found in WVD, could be caused by a difference in thermoregulatory heat loss capacity before habitual sleep onset. We have confirmed these controlled laboratory findings in a week-long ambulatory study. Under real life conditions, WVD showed a lower DPG throughout the day and most relevantly in the evening before sleep onset, together with a prolonged SOL (28).

In addition to the circadian phase difference between WVD and CON, the time course of diverse phase markers were also analyzed with respect to circadian amplitude and 24-h mean level. As shown in Figure 1 not all measured circadian markers show a simple phase delay, as salivary melatonin does. E.g. CBT exhibits in addition to a phase delay (as measured by cross-correlation analysis, Table 5) a tendency to an increase in 24-h mean level. DPG, a measure of distal vasodilatation and heat loss, shows an even more complex pattern. On the first day, 8 h before lights off (which corresponds most closely to real life conditions), DPG was markedly reduced in WVD compared with CON, however, this difference decreases during the following sleep episode. This finding demonstrates the functional vascular disorder in WVD, i.e. vasospasms disappear during the night sleep episode but reappear the next morning. This new vasoconstriction disappears completely during the course of the subsequent CR (see Figure 1). In other words, in comparison to the relaxed state of a CR WVD exhibit an increase in the diurnal amplitude of DPG during normal life. This could be caused by e.g. an increased activity of the sympathetic branch of the autonomous nervous system. It is well known that the sympathetic innervation of the vascular muscles located in distal arterioles and arteriovenous anastomoses is the main determinant of distal skin blood flow, and hence body heat loss. Therefore, the difference between distal skin blood flow in WVD and CON could be caused by changed sympathetic nervous activity.

In previous studies under controlled CR conditions it has been shown that the homeostatic aspect of sleepiness and sleep regulation does not affect the thermoregulatory system (34). In this study no significant differences between WVD and CON could be found in the homeostatic aspect of subjective ratings of

sleepiness (KSS) suggesting, conversely, no influences of the thermoregulatory system on the long-term build-up process of sleepiness. First analyses of the sleep EEG before and after the 40-h sleep deprivation reveals no differences between WVD and CON with respect to slow wave sleep (sleep stages 3 and 4) and slow wave activity (SWA) (39). This would indicate no differences in sleep pressure between WVD and CON. However, further detailed analysis of the build-up and decay rates of SWA is necessary to draw final conclusions (18).

Because the study subjects were measured during their luteal phase it is possible that the described effects are different during the follicular phase. It is well known that estrogens and gestagens exhibit specific effects on the thermoregulatory system. E.g. progesterone increases sympathetically mediated vasoconstriction (13). In a recent survey women with vasospastic syndrome reported that cold hands and feet were not limited to their luteal phase, indicating rather an independence of VS of hormonal status (38) (data not shown). To draw a final conclusion regarding a hormonal influence on the thermoregulatory system in WVD a replication study during the follicular phase would be needed.

Perspectives and significance

One implication of our results is that if WVD would delay their bedtimes sufficiently – by at least 1 h – they would have less or little trouble falling asleep. However, most people usually have constraints on their schedules that necessarily require wakening between 0600 to 0800 h most mornings. A delayed bedtime would result in even less sleep than usual and be less desirable than suffering from DIS. More reasonably, manipulation of the circadian temperature rhythm by resetting the phase position to earlier could alleviate the vasospasms prior to sleep-onset and concomitant difficulties initiating sleep in WVD. Furthermore, we presently investigate whether VS or DIS alone induces the observed differences between WVD and CON leading to information how the thermoregulatory system and sleep initiation interacts.

If a phase advance of the circadian rhythm can normalize DIS in WVD, it will provide a potential non-pharmacological therapy to shift the endogenous rhythm using the appropriate stimulus at the right time (e.g. temperature, light, melatonin). Additional and beneficial effects of administering melatonin to WVD, besides its phase shifting properties (3, 35, 50), would be its ability to induce distal vasodilatation (36) and its

actions on the sympathetic nervous system (2, 11, 50, 52). Another way to relieve WVD of their clinical symptoms is to focus on the putative increased influence of sympathetic activity in this population. That could be done through relaxation techniques such as suggestion of warmth (31), autogenic (25) and biofeedback training (25, 44).

More knowledge of human circadian thermoregulatory function can be of future relevance for the simple treatment of disorders related to a circadian disturbance such as delayed sleep phase syndrome, non-24 h sleep/wake disorder, shift work sleep disorder, jetlag, extreme M/E-types, and social jetlag.

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CHAPTER 3

SLEEP EEG CHARACTERISTICS IN WOMEN WITH DIFFICULTIES INITIATING SLEEP AND VASCULAR DYSREGULATION

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ABSTRACT

Alterations in internal phase angles (e.g. between body temperature rhythms and the sleep-wake cycle) and thermal interventions have been reported to influence sleep onset latency (SOL), and ultradian NREM-REM sleep cycle durations. We have recently shown that women with a vascular dysregulation and difficulties initiating sleep (WVD) show a phase delay in circadian thermoregulation with respect to their habitual sleep-wake cycle in comparison to controls (CON). Therefore, we examined whether WVD also show a different ultradian NREM-REM sleep cycle duration. 17 healthy women (N=8 WVD; 9 CON; luteal phase; age: 20-33yr) performed a 40 h constant routine protocol, with a baseline and a recovery 8 h night before and after. ANOVAs were performed on log-transformed values. In comparison to CON, WVD exhibited a significant longer SOL (WVD 14.9 ± 2.4 min [mean ± SEM]; CON 7.1 ± 1.1 min; $P < 0.01$), a shorter first NREM-REM sleep cycle duration (68.9 ± 4.7 min; 90.2 ± 7.9 min; $P = 0.01$) and a shorter first NREM sleep episode duration (55.7 ± 3.7 min; 75.1 ± 7.4 min; $P = 0.01$). Additionally, WVD tended ($P = 0.09$) towards a shorter REM sleep latency. In WVD the progressive decrease of EEG delta activity (0.75 – 2.0 Hz) across NREM-REM sleep cycles was less pronounced. WVD showed a higher EEG delta activity in the second cycle relative to the first cycle ($P < 0.04$). We conclude that a change in internal phase of entrainment in WVD (i.e. phase delayed thermoregulatory heat loss with respect to the sleep-wake cycle) may influence not only SOL but also ultradian sleep patterns.

INTRODUCTION

There has been considerable evidence that the thermoregulatory system has strong repercussions on sleep regulation. Thermophysiological manipulations prior and also during sleep are able to influence sleep onset latency (SOL), sleep architecture and sleep EEG power spectra (for reviews see (34, 59)).

Sleep in humans usually occurs on the declining portion of the CBT curve when its rate of change, and body heat loss, are maximal (12, 41). Thus, sleep induction and body heat loss exhibit a close temporal relationship. Distal vasodilatation and hence heat redistribution from the core to the shell represents the main determining component of the well-orchestrated circadian down-regulation of CBT in the evening (4, 41). Before lights off, distal vasodilatation is associated with sleepiness and a rapid onset of sleep (10, 30, 41, 42, 47).

Not much is yet known about sleep EEG alterations (sleep architecture and sleep EEG power density) in humans with different thermophysiological properties. Thus, in a series of experiments we have been studying women with a primary vascular dysregulation (VD) – (i.e. 'primary vasospastic syndrome') - under ambulatory and stringently controlled laboratory conditions. VD has been described as a functional vascular dysregulation in otherwise healthy subjects, whose main symptom is thermal discomfort from cold extremities (hands and feet) (27). In other words, subjects with VD can be characterized as having a disposition for a thermophysiologically large shell and small core, providing therefore a model of nature to study the relationship between thermophysiology and sleep without any invasive manipulations.

In a representative survey in the Canton Basel-Stadt we could recently show that up to 30% of women but only 7% of men between 20 and 40 years exhibit VD (43). The relative risk for difficulties initiating sleep (DIS, a symptom belonging to the DSM-IV criteria for insomnia (1)) is more than doubled in these subjects, independent of gender. In another study under real life ambulatory conditions we could show that women with both primary vascular dysregulation and DIS (WVD) exhibit a phase delay of distal skin temperature rhythms with respect to their sleep-wake cycle in comparison to controls (CON) (33). This resulting shift in internal phase of entrainment between the thermoregulatory system and the sleep-wake cycle could be confirmed also under very stringent constant routine conditions in the laboratory (60).

Here we analysed sleep EEG recordings from 8-h sleep episodes before and after a 40-h sleep deprivation from the same study mentioned above (60). We aimed at characterizing the sleep EEG in WVD and CON by means of stage- and EEG power spectral analyses with respect to the influence of circadian, homeostatic, and thermal factors. Does the different thermophysiological condition of WVD, i.e. their diathesis of enhanced heat retention, hence reduced heat loss, alter their sleep architecture? The temperature differences between WVD and CON are evident before lights off and fade away during subsequent sleep, therefore, assuming a direct effect of temperature on sleep, we would expect a similar pattern in sleep structure, i.e. sleep should be more affected around sleep onset and in the first part of the sleep episode.

METHODS

Subjects

Two groups of healthy young women (WVD N = 9, and CON N = 9) were recruited via poster advertisements at the University of Basel and via announcements on the internet (for their physiological characteristics see Table 1). They had so successfully complete the Torsvall-Åkerstedt morning-evening-type questionnaire (57) and two questionnaires covering sleep habits, sleep quality, life habits, physical health, medical history, and thermophysiological behavior. Exclusion criteria were extreme morning or evening types (ratings ≤14 and ≥21 points), , chronic or current major medical illness or injury, amenorrhea or irregular menstrual cycles, smoking, intake of over-the-counter or prescription medications (including oral contraceptives or other hormonal treatments) or illicit substances, shift work within 3 months or transmeridian travel within 1 month prior to the study, excessive caffeine (i.e. > 300mg) and alcohol consumption (i.e. > 1 beverage per day).

Potential study participants who fulfilled these criteria reported to the University eye clinic for a finger nailfold video capillary microscopy to objectively confirm self-ratings about cold or warm extremities, respectively (inclusion criteria: blood standstill for ≥ 12 sec = WVD, < 12 sec = CON according to (27, 31). Additionally, the nailfold skin temperature was measured. After a physical examination to exclude any medical disorders, a polysomnographically recorded screening night in the laboratory was performed to test the participants' ability to sleep in a new environment, to exclude primary sleep disorders (i.e. insomnia) and to assess sleep onset latency to sleep

stage 2 (≥ 20 min for WVD, < 15 min for CON, see Table 1). Participants with a sleep efficiency of lower than 80% were excluded from participation.

Selected participants entered the study prior to the first but after the fourteenth day of their menstrual cycle in order to complete the experiment within the luteal phase. During 7 days before their admission to the laboratory (baseline week) subjects were instructed to maintain a regular sleep-wake schedule (bedtimes and wake times within ± 60 min of self-selected target times scheduled 8 h apart). Adherence to this regular schedule was verified with a wrist activity monitor (Cambridge Neurotechnologies®, UK) and sleep-wake logs. They were also instructed to abstain from excessive caffeine and alcohol consumption as well as heavy physical exercise. The design, purpose, and the risks of the study were explained prior to the written consent of the participants. It was explicitly permitted to stop the experiment at any time. The study protocol was approved by the local ethical committee ('Ethikkommission beider Basel') and conformed to the guidelines contained within the Declaration of Helsinki. All 18 participants completed the study without any complaints.

Study design and protocol

After the baseline week, the volunteers reported to the laboratory 2 h before their habitual bedtime for an adaptation night (the timing of their sleep–wake schedule was calculated in such way that the sleep episode was centered at the midpoint of each subject's habitual sleep episode as assessed by actigraphy during the baseline week). They were prepared for continuous polysomnographic and temperature recording. Subjects were allocated to a sound attenuated, air-conditioned chronobiology room controlled for light (< 8 lux [typically 3-5 lux at the angle of gaze] during wakefulness and 0 lux during scheduled sleep), ambient air temperature (22℃) and relative humidity (55%). After the 8-h s leep episode (adaptation night) the subjects were adjusted to the experimental dim light conditions (< 8 lux). They were allowed to walk around in the Chronobiology facility. To assure that the light levels never exceeded 8 lux, participants were asked to wear sunglasses. In the afternoon subjects lay down exactly 30 minutes before the start of the protocol (8 h before lights off). Subjects were covered with a blanket but could adjust their bedcovers to maintain thermal comfort. Isocaloric snacks were given hourly and water was

available ad libitum. After a second 8-h sleep episode (baseline night [BL]), the subjects followed a 40-h CR with constant wakefulness and a subsequent third 8-h sleep episode (recovery night [RE]). The study was completed 2 h after waking up from RE.

Physiological measurements

Sleep recordings

Sleep episodes were polysomnographically recorded using the Vitaport Ambulatory system (Vitaport-3 digital recorder TEMEC® Instruments B.V., Kerkrade, The Netherlands). Six EEG derivations (frontal [Fz], central [Cz, C3, C4], parietal [Pz], occipital [Oz], referenced against linked mastoids, A1, A2), two electrooculograms, one submental electromyogram, and one electrocardiogram were recorded. All EEG signals were filtered at 30 Hz (fourth-order Bessel-type anti-aliasing low-pass filter, total 24 dB/octave), and a time constant of 1.0 second was used prior to online digitization (range 610 µV, 12 bit analog-to-digital converter, 0.15 µV/bit; storage sampling rate at 128 Hz for the EEG). The raw signals were stored online on a Flash RAM Card (Viking, Rancho Santa Margarita, Calif) and downloaded offline to a personal computer hard drive.

Thermometry

Temperature data were continuously recorded by a computerized system (System Hofstetter, SHS Allschwil, Switzerland) in 20-sec intervals, as previously described in detail (60). Rectal temperature and skin temperatures from 8 sites were measured (for details see (60)). For correlational analyses with EEG parameters the distal-proximal skin temperature gradient (DPG) was calculated (60).

Data Analysis

EEG stage analysis

All sleep episodes were visually scored (Vitaport Paperless Sleep Scoring Software; TEMEC® Instruments) for consecutive 20-s epochs (C3-A2 derivation) according to standard criteria (37, 53). SOL was defined as the time interval between lights off and the occurrence of the first 20-sec epoch of sleep stage 2. REM sleep latency (REML) was calculated from sleep onset (defined as latency to stage 2). All sleep latencies

(latency to stage 1[SL1], latency to stage 2 [SL2], latency to stage 3 [SL3], latency to stage 4 [SL4], REML) were log - transformed before statistical analysis. Total sleep time (TST) was defined as Stage 1 + 2 + 3 + 4 + REM sleep. Sleep efficiency (SE) was defined as follows: SE = TST/time between lights off and lights on x 100. Wakefulness after lights off (WALO; % of TST) and wakefulness after sleep onset (WASO; % of TST) were also measured. Non-rapid eye movement sleep (NREMS) was defined as stages 2 to 4 (% of TST). Sleep stages (1-4), rapid eye movement sleep (REMS), wakefulness, and movement time (MT) were expressed as percentage of total sleep time (TST) during the respective night for all participants (Σ stages 1-4, REMS). TST and sleep latencies and REML are indicated in minutes.

EEG spectral analysis

EEGs were subjected to spectral analysis using a fast Fourier transform (10% cosine 4-s window), resulting in a 0.25 Hz bin resolution. EEG artifacts were detected by an automated artifact detection algorithm (Vitascore, CASA; 2000 Phy Vision B.V., Kerkrade, The Netherlands). For final data reduction, the artifact-free 4-s epochs were averaged over 20-s epochs and matched with the 20-s epochs of the visual sleep scoring.

We report EEG data of NREMS derived from the midline (Fz, Cz, Pz, Oz) in the frequency range of 0.75-25 Hz, and averaged for six frequency bands: slow-wave activity (SWA; EEG power density in the range of 0.75-4.5 Hz), theta (4.5-8 Hz), alpha (8-12 Hz), low sigma (12-14 Hz), high sigma (14-16 Hz), beta (16-25 Hz).

Sleep cycle analysis

NREM-REM sleep cycles were defined according to the criteria of Feinberg and Floyd (24), with the exception that, for the last sleep cycle analyzed (cycle 4), no minimum REMS duration was required. For cycles 2-4, the first 20-s epoch after the last REMS episode was defined as the onset of a cycle. All participants completed at least 4 sleep cycles, therefore the first 4 cycles were subjected to further analysis

Statistical analyses

The statistical packages StatView™ 5.0 and SuperANOVA™ (Abacus Concepts, Berkeley, California, USA), and STATISTICA 6 ™ for Windows (StatSoft Inc., Tulsa, USA) were used. Analyses of time courses were performed by cross-correlation analyses and by two-way rANOVA with grouping factor *group* (WVD, CON) and

repeated factor *time* (BL, RE). All *P* values derived from rANOVAs were based on Huyhn-Feldt corrected degrees of freedom, but the original degrees of freedom are reported. For *post-hoc* comparisons Fisher's PLSD with alpha-correction for multiple comparisons according to Curran-Everett (14) were calculated. For statistical analyses between WVD and CON without an a priori hypothesis, the threshold for alpha-errors was set at $P < 0.05$ (two-sided, not especially indicated), otherwise at $P < 0.1$ (one-sided, indicated by †). The Mann-Whitney U-test was used to reveal significant differences between WVD and CON. Means ± SEM values are presented.

RESULTS

Characteristics of subjects

Table 1 presents the descriptive and inferential statistics for age, body mass index (BMI), finger temperature, and data from the sleep/wake diary (including actimetry), sleep questionnaires and polysomnographic recordings of SOL to sleep stage 2. WVD and CON did not significantly differ in age, BMI and sleep times, but their objectively measured finger temperatures were significantly lower while sleep latency was significantly longer (for statistics see table 1). Thus, the subjective rating of difficulties initiating sleep in WVD during the screening week was polysomnographically confirmed by significant longer sleep onset latencies in both the screening and adaptation nights.

Table 1. Physiological characterization of the study participants

Variable	CON (N=9)	WVD (N=9)	*P* value (U-test) CON vs. WVD
Age, years	25.1 ± 1.7	24.2 ± 1.2	0.50
BMI, kg/m^2	20.85 ± 0.6	20.82 ± 0.54	0.96
Fingertemp, ℃ (nailfold skin temp. on screening)	32.83 ± 0.49	28.5 ± 0.99	**0.002** †
Sleep times baseline week (sleep-wake logs)			
Lights off	23:46 ± 0:07	23:25 ± 0:12	0.20
Lights on	07:44 ± 0:07	07:24 ± 0:12	0.21
SOL baseline week, (sleep-wake logs), min	15.02 ± 3.27	31.59 ± 4.46	**0.0025** †
SOL screening night, polysomnography, min	10.04 ± 1.14	37.41 ± 10.47	**0.0001** †
SOL BL, polysomnography, min	8.82 ± 1.24	19.11 ± 3.54	**0.01** †
SOL RE, polysomnography, min	5.37 ± 1.13	9.78 ± 1.48	**0.015** †

Values are means ± SEM. Values for reported sleep times are in hours:minutes. Polysomnographically obtained sleep onset latency (SOL) refers to the interval between lights out and the first epoch of sleep stage 2.

Sleep stages

Table 2 summarizes the sleep parameters calculated over the entire sleep episode for BL and RE. As expected due to the selection criteria (see methods) the SL to stage 2 revealed a significant main *group* effect ($F_{1,16}$ = 2.99; P = 0.01) as did SL to stage 3 and 4 ($F_{1,16}$ = 6.83; P = 0.02 and $F_{1,16}$ = 5.54; P = 0.03, respectively) without any significant *group x night* interaction. REML tended towards significance for the main effect *group* ($F_{1,15}$ = 3.29; P = 0.08), i.e. a shorter REML in WVD. The number of minutes of NREMS, of SWS and percentages of SWS showed a significant main effect of *night* ($F_{1,16}$ = 56.4; P < 0.0001 for both NREMS and SWS). Interestingly, the significant *group x night* interaction term ($F_{1,16}$ = 5.12; P = 0.03) indicated that WVD exhibit a more pronounced increase of SWS from BL to RE.

Table 2. Sleep variables calculated for Baseline Night (BL) and Recovery Night (RE) of WVD and CON.

Sleep variable	Group	Night	Total night	Cycle 1	Cycle 2	Cycle 3	Cycle 4
TST	CON	BL	450.3±4.7				
	WVD	BL	442.5±6.7				
	CON	RE	460.4±4.1				
	WVD	RE	459.1±4.1				
SE %	CON	BL	93.7±1.0				
	WVD	BL	92.2±1.4				
	CON	RE	95.6±0.8				
	WVD	RE	96.3±0.6				
SL 1	CON	BL	6.6±1.0				
	WVD	BL	15.2±3.8 (*****)				
	CON	RE	4.2±1.0				
	WVD	RE	6.5±1.7 (*****)				
SL 2	CON	BL	8.8±1.2				
	WVD	BL	**19.1±3.5 ***				
	CON	RE	5.4±1.1				
	WVD	RE	**9.8±1.5 ***				
SL 3	CON	BL	19.1±1.9				
	WVD	BL	**29.2±3.6 ***				
	CON	RE	10.2±1.3				
	WVD	RE	**14.5±1.8 ***				
SL 4	CON	BL	22.9±2.2				
	WVD	BL	**41.1±8.0 ***				
	CON	RE	15.1±1.4				
	WVD	RE	**17.9±1.8 ***				
REML	CON	BL	76.7±12.1				
	WVD	BL	51.8±3.8 (*)				

REML	CON	RE	73.5±9.3				
	WVD	RE	59.6±6.3 (*)				
arousal	CON	BL	68.5±7.9	4.7±2.2	7.5±1.6	11.1±3.0	12.0±2.7
	WVD	BL	77.2±7.4	3.1±1.1	6.8±1.4	10.6±0.6	12.9±1.9
	CON	RE	37.8±6.7	2.8±0.8	4.6±1.9	5.0±1.1	6.7±1.8
	WVD	RE	43.0±8.8	0.9±0.4	4.5±1.4	6.3±1.6	10.4±2.9
stage 2	CON	BL	224.9±5.	31.7±5.7	37.3±3.1	46.7±3.7	50.0±3.0
	WVD	BL	233.3±5.1	**22.6±2.1** †	36.0±3.3	62.1±5.1	55.3±4.0
	CON	RE	229.6±7.0	23.8±4.3	40.9±4.3	48.3±5.1	49.6±3.1
	WVD	RE	223.3±11.0	**17.0±3.5** †	33.5±6.9	55.7±5.3	43.7±4.5
SWS	CON	BL	93.0±7.1	43.1±6.1	21.2±3.8	15.4±4.3	6.7±2.9
	WVD	BL	**77.1±9.5 #**	28.0±5.4	28.7±4.9	10.2±2.9	3.8±2.3
	CON	RE	124.6±9.2	54.5±6.6	35.9±8.3	20.6±4.0	10.4±3.
	WVD	RE	**125.9±11.7 #**	43.5±4.6	45.9±6.3	20.1±5.5	9.3±5.1
NREM	CON	BL	318.0±6.4	74.8±10.3	58.5±3.0	62.1±3.7	56.7±2.8
	WVD	BL	310.3±7.5	**50.6±3.9** †	64.6±3.8	72.4±3.6	59.1±4.4
	CON	RE	354.3±9.2	78.3±9.1	76.7±6.7	68.9±5.5	60.0±3.5
	WVD	RE	349.2±12.	**60.5±6.7** †	79.4±6.0	75.8±6.0	53.0±5.4
REM	CON	BL	94.0±6.5	8.5±2.3	15.9±2.1	22.1±3.4	22.7±5.2
	WVD	BL	92.3±5.7	12.3±2.1	16.2±2.7	19.0±2.2	20.9±3.3
	CON	RE	86.9±4.3	11.2±2.0	13.4±1.4	16.4±2.7	20.4±2.7
	WVD	RE	83.8±7.2	9.5±2.5	12.9±2.3	17.0±4.0	18.6±2.6
SWS %	CON	BL	20.6±1.4	50.0±4.1	26.8±5.8	16.6±4.8	8.4±4.2
	WVD	BL	**17.3±2.1 #**	40.8±6.7	32.7±5.6	10.6±3.2	4.1±2.0
	CON	RE	27.0±1.8	59.9±3.1	35.8±7.8	23.2±5.1	11.7±3.3
	WVD	RE	**27.3±2.4 #**	61.7±4.3	48.9±6.6	20.3±5.2	10.9±5.9
NREM %	CON	BL	70.7±1.5	85.9±2.8	72.2±3.2	66.9±4.2	64.5±5.7
	WVD	BL	70.2±1.4	77.2±3.5	73.6±3.5	70.9±1.9	63.7±3.6
	CON	RE	76.9±1.6	84.9±2.7	81.0±3.1	77.0±3.1	69.8±3.4
	WVD	RE	76.0±2.3	85.0±3.2	82.2±2.5	76.6±2.8	64.1±4.9
REM %	CON	BL	20.8±1.3	9.9±2.3	19.0±2.0	22.2±2.3	22.9±4.1
	WVD	BL	20.8±1.2	18.2±2.5	18.4±3.0	18.5±1.8	22.4±3.5
	CON	RE	18.9±1.0	12.1±2.4	14.1±1.2	17.5±2.2	22.7±2.0
	WVD	RE	18.3±1.6	12.4±2.7	13.2±2.2	16.4±3.5	23.0±3.1

Sleep stages derived from visual scoring for WVD (N = 8) and CON (N = 9) for baseline (BL) and recovery (RE) night (mean ± SEM). TST refers to total sleep time (= stage1 + 2 + 3 + 4 + REM sleep); SE, sleep efficiency (=(TST/time in bed) x 100); arousal = wakefulness after lights off + movement time + stage 1; SL, latency to stage 1 (min); SL2, latency to stage 2 (min); SL3, latency to stage 3 (min); SL4, latency to stage 4 (min); REML, latency to REM sleep (min). For SL1, SL2, SL3, SL4, and REML, statistics were applied on log-transformed values. Values are in minutes or in percentage of TST. ***P<0.05, BL, WVD + RE, WVD > BL, CON + RE, CON;*** ***P<0.1, BL, WVD + RE, WVD > BL, CON + RE, CON;*** *#P<0.05:RE, WVD - BL, WVD > RE, CON -; BL, CON; †P<0.05, Group x Cycle: WVD vs CON x BL vs RE.*

NREM-REM sleep cycle durations

A three-way rANOVA with the factors night, group, and cycle was performed. As the interaction terms *night x group x cycle* and *night x group* revealed no significance, BL and RE were pooled for further analyses and data presentation, see Figure 1. A significant *group x cycle* interaction term was found for the duration in minutes of stage 2 ($F_{1,3}$ = 4.17; P = 0.01), as WVD showed a shorter stage 2 duration in the first cycle compared to CON. Similarly, the amount of NREMS revealed a significant *group x cycle* interaction term ($F_{1,3}$ = 3.69; P = 0.02) , since WVD had significantly less NREMS in the first sleep cycle compared to CON. The analysis of the duration of the NREM-REM sleep cycles revealed a significant *group x cycle* interaction term for NREM-REM sleep cycle and NREMS duration ($F_{1,3}$ = 2.87; P = 0.04, and $F_{1,3}$ = 3.40; P = 0.03, respectively), with a shorter first NREM-REM sleep cycle duration and a shorter NREMS duration in the first cycle in WVD (see Figure 1: NREM-REM sleep cycle 1 duration: WVD (N = 8), 68.9 ± 4.67 min [mean ± SEM]; CON (N = 9), 90.2 ± 7.91 min; posthoc unpaired *t*-test, P = 0.01; NREMS duration: WVD = 55.7 ± 3.68 min; CON 75.1± 7.42 min; posthoc unpaired *t*-test, P = 0.01). Within WVD a significantly shorter duration of the first cycle compared with cycle two, three, and four (P < 0.03) was found, a pattern that could not be observed in CON.

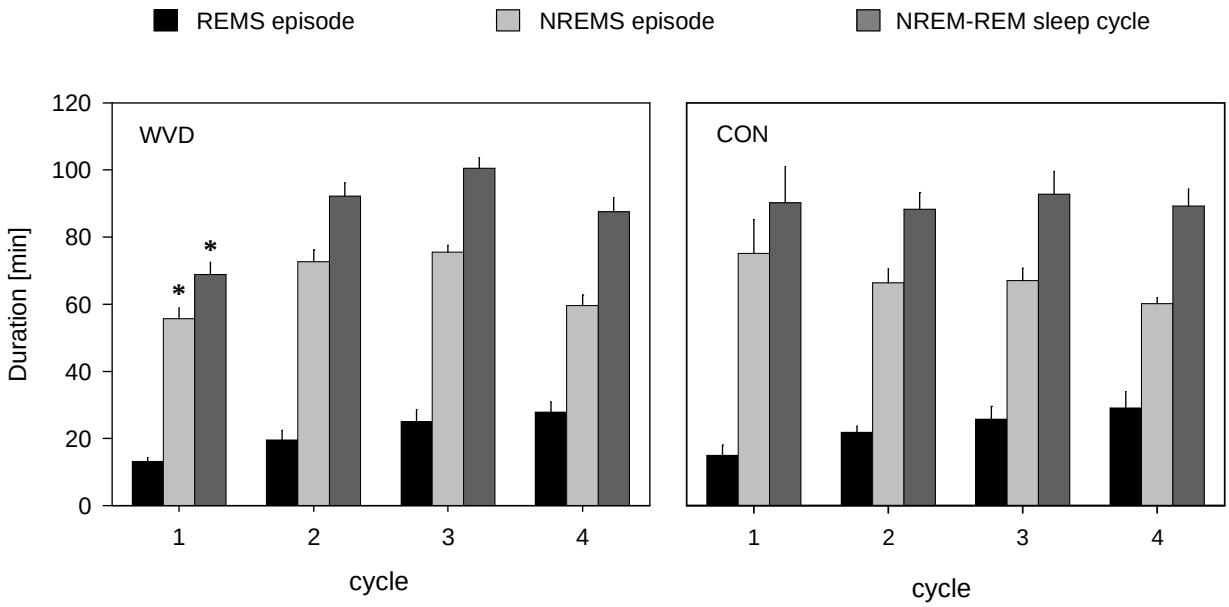

Figure 1. *Duration (mean + SEM) of rapid eye movement sleep (REMS), non-REMS (NREMS), and duration of NREM-REM sleep cycle in the first 4 NREM-REM sleep cycles in WVD (N=8, left panel) and CON (N=9, right panel). *P < 0.05 WVD vs CON (unpaired t-test).*

EEG Power Density

Due to no, or only minor, effects of the 40 h sleep deprivation episode on differences between WVD and CON in the EEG power density spectra (see below), BL and RE data were pooled.

EEG power spectra of the entire sleep episode

EEG power density spectra in the frequency range of 0.25-25 Hz in WVD relative to CON is illustrated in Figure 2a. In general, WVD tended to have lower EEG power density in some of the frequency bins in the alpha frequency range (unpaired *t*-test, p < 0.1). A separate unpaired *t*-test for each derivation revealed tendencies of a group difference in some frequency bins in the alpha range of the Cz derivation, and in the delta and alpha range of the Oz derivation (*P* = 0.1 for each frequency bin). That is, compared to CON, WVD tended towards a decreased power density in those frequency bins.

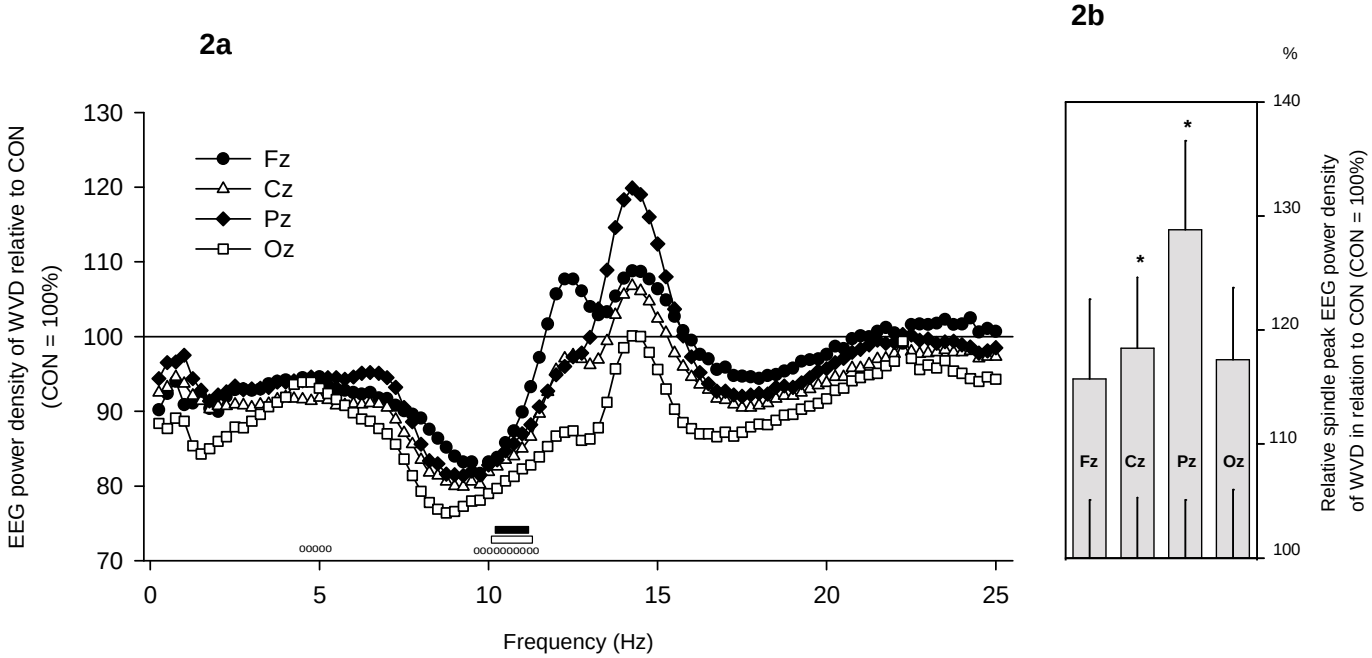

***Figure 2a**. EEG power density during NREM sleep in the frequency range of 0.25-25 Hz for all-night in WVD (n = 9) relative to CON (n = 9) separately for midline derivations (Fz, Cz, Pz, Oz). Horizontal black bars at the bottom of the panel indicate frequency bins for which the factor group revealed tendency (two-way rANOVA, P < 0.1). Open bars indicate frequency bins for which the value for Cz in WVD tended to be different from the value of CON, open circles indicate frequency bins for which the value for Oz in WVD tended to be different from the value of CON (unpaired t-test on log transformed values, P < 0.1).*

***Figure 2b**. Relative spindle peak of WVD relative to CON during all-night between 8.25 to 16.0 Hz. Relative spindle peak was calculated for each individual by subtracting the averaged frequency bins of 8.25 - 9.0 Hz and 15.25 - 16.0 Hz from the values of the 14.0 - 14.25 Hz bin. Values are means +/- SEM. *P < 0.04; unpaired t-test.*

Visually, in Figure 2a, the pattern of the curve of WVD relative to CON in the sigma frequency range indicates possible differences in the size of the relative EEG activity in the sigma frequency range. Therefore, to quantify possible differences between CON and WVD, a relative spindle peak of WVD relative to CON was calculated (for details see legend to Figure 2b). A two-way rANOVA (*group* and *derivation*) revealed a significant main effect of *group* ($F_{1,16} = 5.8$; $P < 0.03$) and significance for the *group x derivation* interaction ($F_{1,3} = 3.8$; $P < 0.02$). WVD exhibit a significantly larger relative spindle peak than CON. This difference was significant in the Pz and Cz derivation (unpaired *t*-test, $P < 0.01$ and $P < 0.04$, respectively) and tended towards significance in the Fz and Oz derivation ($P < 0.1$).

Temporal evolution of EEG power spectra of the first 4 NREM-REM sleep cycles

Figure 3 illustrates relative NREM sleep EEG power densities in WVD (CON=100%) across the first 4 NREM-episodes for each derivation separately in between 0.75-25 Hz.

Two-way rANOVAs of each derivation were performed. Significant interactions (*group x cycle*) were found for some 0.25Hz-bins in the delta –range of the Pz and Oz derivation ($P < 0.04$) and tendencies ($P < 0.1$) towards significance in Fz and Cz. In all these derivations WVD exhibited higher relative delta EEG power density values in the second cycle in relation to cycle 1, 3 and 4 than CON (two-way rANOVAs (*group x cycle (cycle1,3,4 vs. cycle 2)*, all derivations $P < 0.02$).

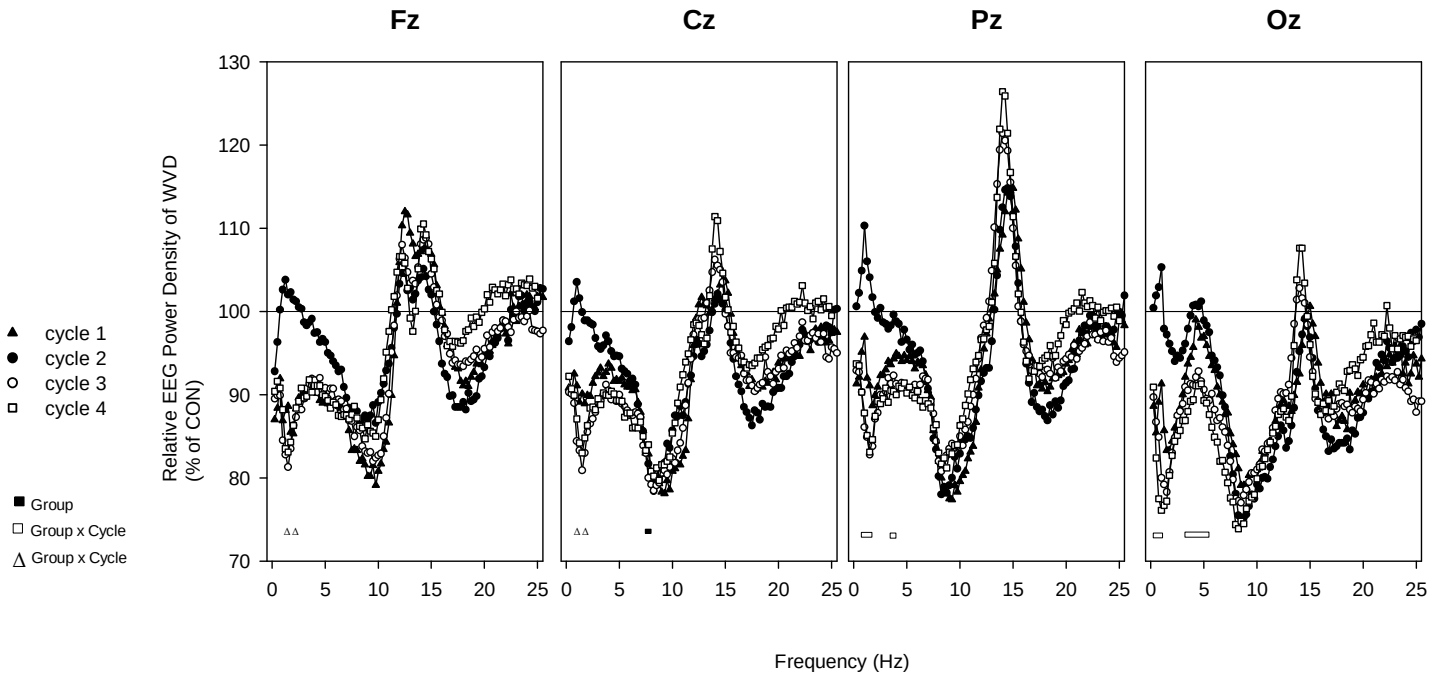

Figure 3. *EEG power spectra averaged across BL and RE of WVD (N = 8) for midline derivations (Fz, Cz, Pz, Oz) expressed relative to values of CON (N = 9), separately for sleep cycles. Open and filled bars at the bottom of the panel indicate frequency bins for which the factor group and the interaction term group x cycle was siginificant (3-way rANOVA, P < 0.05). Open triangles indicate frequency bins for which the interaction term group x cycle showed tendency (P < 0.1).*

In a further analysis, the temporal evolution of EEG power spectra of successive NREM-REM sleep cycle power densities were calculated relative to cycle 1. Although in both groups power density of the delta band progressively decreased over consecutive NREM-REM sleep cycles, the decrease in WVD was less pronounced for the second cycle (data not shown). Thus, WVD have a higher power density in the delta frequency range in the second sleep cycle relative to cycle 1 than CON, underpinning the previous analyses.

To examine whether the build-up of SWA within the first 30 minutes of each NREMS episode differed between the two groups, the rise rate of SWA was calculated from the median slope of adjacent 1-minute epochs for BL and RE for Fz. The mean values of the median slopes were subjected to unpaired *t*-tests. A 3-way rANOVA with the factors *night*, *cycle*, and *group* revealed no significance indicating no differences between WVD and CON in the build-up of SWA.

Effect of sleep deprivation on the EEG power spectra

In order to visualize the repercussions of 40-h of sleep deprivation on the sleep EEG, EEG power density obtained during RE was expressed as a percentage of the

corresponding value from BL (=100%) for both WVD and CON (Figure 4). Data are shown exemplarily for Fz (other derivations revealed similar findings). A 2-way rANOVA with factors *group* and *night* revealed a significant main effect for the factor *night* in the frequency ranges from 0.5-12.0 Hz, 14.5-15.25 Hz, and 17.25 – 25.0 Hz ($P < 0.05$ in all cases). Significant interactions between the factors *group* and *night* were found for frequency bins in the beta range ($P < 0.05$). Compared to CON, WVD display higher EEG power density in the beta frequency range in the night after SD with an otherwise similar response.

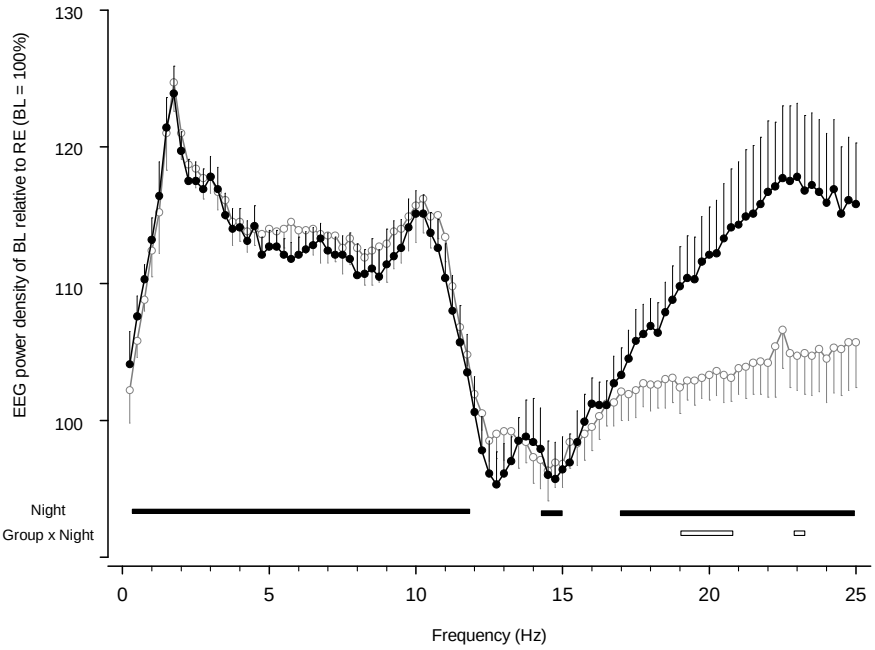

Figure 4. *Effects of sleep deprivation on the power spectra in NREMS for Fz. Data of RE were expressed as percentage (± SEM) of the corresponding value in BL. Black and open bars at the bottom indicate frequency bins for which the factor night and the interaction term group x night was significant (2-way rANOVA, P < 0.05).*

Correlation between the first NREMS episode duration and DPG 30 min before lights off

To test whether there was a relationship between the shortened first NREMS episode and DPG, inter-subject Pearson-regression analysis was performed. Pooled DPG-values (℃) of the 0.5-h period before lights off and the duration of the first NREMS episode (min) was used for the analysis (Figure 5). A significant correlation was found between DPG and the first NREMS episode.

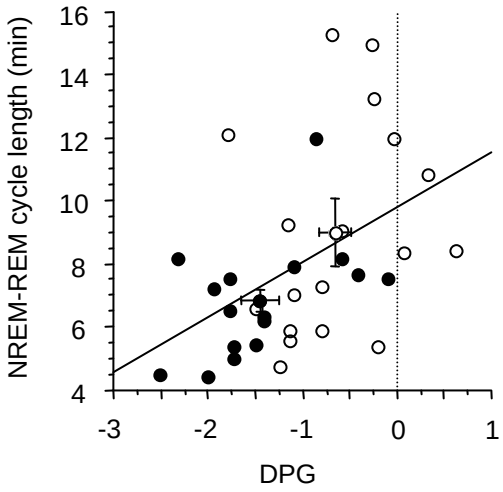

Figure 5. *Correlation between distal-proximal gradient (DPG) (mean value -0.5 - 0 h before lights off) and 1st NREM-REM sleep cycle duration. Mean values of BL and RE of each subject (N = 34) are shown. Filled circles depict data of WVD (N = 8), open circles of CON (N = 9). Additionally, mean values ± SEM are shown for WVD and CON. The black line represents the regression line.*

Taken together, sleep stage and sleep EEG power spectral analyses disclosed specific differences between WVD and CON. During the entire 8 h sleep episodes (BL and RE) WVD revealed a slightly reduced power density in the delta frequency range (Figure 2a), which is in accordance with the reduced amount of SWS during BL (Table 2). WVD also showed a reduced power density in the alpha frequency range, which contributes to the calculated larger relative spindle peak (Figure 2b). In both BL and RE, WVD not only showed longer SOL (Table 1), but also a shorter first NREM-REM sleep cycle (Figure 1). In addition to these differences, WVD revealed specific changes in the evolution of certain components of the EEG power spectrum within a nocturnal sleep episode. For example, delta power density in WVD did not follow the usual progressive decrease over consecutive NREM-REM sleep cycles (Figure 3) – the decrease was less pronounced from the first to the second cycle.

Challenging the sleep homeostat exhibited an intact regulation in WVD. A 40-h sleep deprivation reduced their SOL (but still longer than CON, Table 2) and increased the amount of SWS to a similar level as in CON (Table 2). The higher sleep pressure induced a similar increase of EEG power density in the delta, theta, and alpha frequency ranges as in CON (Figure 4).

DISCUSSION

Our sleep analyses revealed noteworthy differences between WVD and CON. Two findings in WVD are of special interest: first the reduced duration of the first NREM-REM sleep cycle because of a diminished first NREMS episode, and second the different evolution of delta power density across successive NREM-REM sleep cycles, i.e. the decrease in delta-power was less pronounced from the first to the second cycle. Are these two findings interrelated?

It is well known that NREMS and REMS are interrelated and that homeostatic, circadian, and ultradian interactions generate a specific NREM-REM sleep pattern in the first and subsequent NREM-REM sleep cycles (7). The empirical observations of an ultradian process occurring within the sleep episode representing the alternation of two basic sleep states NREMS and REMS has been formalized in the interaction model of REM regulation of McCarley and Hobson (35, 48). It can be summarized as follows: REM inhibiting neurons (aminergic RemOff cells) have an inhibitory autofeedback that stops their own activity and allows other neurons (cholinergic RemOn cells) to gain activity and generate REMS evident from the 80-120 min periodicity of the NREM-REM sleep cycle in humans. Another theory advocates a homeostatic regulation described by the 2-process model (5), where any changes of 'Process S' (e.g. NREMS pressure) will impact NREM-REM sleep cycle. The observed shorter first NREMS episode with a slightly shorter REML in WVD could be explained by two possible discrete or combined causes. Namely, by an increased REMS pressure or by a reduced NREMS pressure. However, this cannot be disentangled by the applied protocol.

Nevertheless, the following arguments speak against increased REMS pressure as the causal factor. Increased REM pressure does not necessarily lead to a shorter REML, but rather to longer REMS duration, as demonstrated in REM deprivation studies (6, 11, 32). We found no evidence that WVD had different REMS duration from CON. The timing of REMS is strongly influenced by the circadian pacemaker (15, 16, 19, 64, 66). Since WVD exhibit a phase delay in their circadian system (e.g. CBT) (60), the first REMS episode should have occurred later – which it didn't.

Thus, a higher REMS pressure in WVD seems to be rather unlikely, which in turn favors a decreased NREMS pressure as underlying our results. This interpretation is supported by the following findings. Diverse nap studies could induce changes similar to those we found, e.g. longer SOL, shorter first NREMS episode, shorter REML, no differences in REM duration and reduced delta power density (25) (17, 26, 65). A further similarity to WVD can be observed in the dynamics of delta power density within a post-nap night: the reduction of SWA from NREMS episode 1 to episode 2 is no longer significant. This pattern can be explained as a consequence of the shortened first cycle. The time available for NREMS was not sufficient in WVD to accommodate the entire recovery within the first cycle, thus, homeostatic activity was carried over to the second cycle (18), which can be called “internal rebound”.

There are different kinds of subject groups showing striking similarities with respect to sleep characteristics in WVD. For example, chronic insomniacs exhibit similar changes in NREM-REMS cycle duration and an internal rebound of SWA in the second NREM-REM sleep cycle (44, 49). Therefore, the homeostatic control within a sleep episode seems to be intact in insomniacs. In studies with depressed subjects (44) the pattern of SWA in the first and second sleep cycle also resembles the pattern observed in WVD. Additionally it is reported that depressed subjects have generally less SWS and a reduced REML (45, 54). Furthermore, older individuals represent another group of subjects showing shorter REML with concomitantly reduced SWS (20). Taken together, these subject groups show a certain resemblance to the sleep pattern found in WVD, supporting the notion that reduced NREMS pressure (reduced SWA) at the beginning of sleep reduces first NREMS duration and hence internal rebound from the first to the second NREM-REM sleep cycle.

How can the differences in sleep pattern between WVD and CON be explained? Practically any of the differences observed between CON and WVD could be responsible.

Reduced heart rate variability (HRV), preferentially in the high frequency range, was found in WVD (3) indicating a predominance of sympathetic nerve activity. Psychologically, WVD are characterized as turning their anger/aggression inwards more often than CON (61), which could lead to a higher sympathetic nerve activity.

Whether and how this possible higher sympathetic activity influences the EEG pattern in WVD remains to be elucidated by means of HRV analysis of their sleep. Many studies revealed a strong coupling between cardiac and EEG activities (2, 9, 23). A reduced HRV in WVD during sleep together with the observed tendency towards a reduced SWA would be in accordance with the reported inverse coupling between oscillations in delta waves and HRV (9). And the observed lower alpha activity during NREMS of WVD together with an altered HRV could stand for a lightened NREMS compared to CON (23). Patients suffering from major depression were reported to exhibit a hyperactivity of their hypothalamic-pituitary-adrenal axis (36) with an increased cortisol secretory activity and concomitant sleep alterations as described above (58). It could be possible that in WVD an increased cortisol level in the evening could contribute to the observed altered sleep architecture. The future analysis of the obtained saliva samples will proof this suggestion.

WVD are a selected group with an exclusive diathesis to VD and DIS (i.e. sleep onset insomnia). The number of studies focusing specifically on the subgroup of sleep onset insomniacs (SOI) is scarce. Apart from the longer SOL, SOI exhibit virtually no differences in EEG power spectra from normal sleepers (28), but a phase delay of their CBT rhythm has been observed (50) . Because sleep onset insomnia seems to elicit no remarkable alterations to the sleep pattern after sleep initiation, it is unlikely that a prolonged SOL *per se* is a cause of the altered sleep pattern found in WVD.

However, the amount of heat dissipation preceding sleep correlates best with SOL (42). Therefore heat loss regulatory mechanisms around sleep onset may be crucial for both SOL and the subsequent sleep pattern. One of the striking characteristics of WVD that we have documented is their markedly reduced DPG during subjective day and around the sleep onset period, with this difference disappearing during the first hour of the sleep episode; the same has been reported for SOI (29). More specifically, we observed that the reduced DPG in WVD around sleep onset is due to a 1 h phase delay of the circadian system (e.g. melatonin-, CBT-, DPG-rhythm) with respect to the sleep-wake cycle (33, 60).

Does the altered internal phase angle between the circadian system and the habitual sleep-wake cycle in WVD contribute to the observed sleep pattern? As REMS is tightly coupled to the circadian CBT rhythm (15, 16, 19, 38, 64, 66), phase angle

differences may influence the NREM/REM sleep pattern as observed in WVD. Altered internal phase angles have been reported not only in SOI (50) but in depression (8, 56) and delayed sleep phase syndrome (DSPS) (46, 62, 63), both characterized by longer SOL and disturbed sleep. When DSPS patients are allowed to sleep whenever they feel tired, regardless of time of day, their sleep structure and subjective sleep quality are almost normal (55), however, recent reports suggest that DSPS is not only a disorder of habitual sleep timing but of a generally abnormal circadian timing system (13). Whether this would be the case in WVD is the purpose of an ongoing study by delaying their bedtimes for 1 h or advancing their endogenous circadian rhythm for 1 h.

A close relationship between temperature and the first NREM-REM sleep cycle could be confirmed in this study: DPG correlates positively with the first sleep cycle duration (Figure 5) and with SWA (data not shown, $P = 0.05$). However, a different temperature pattern around sleep onset, i.e., reduced heat loss at the beginning of sleep, may impact on the sleep EEG not only in the first part of the sleep episode but also later on. For example the observed differences in EEG power density in the sigma frequency range, where WVD showed a higher relative spindle peak than CON, seems not to be directly (i.e. at the same time) coupled to thermoregulatory processes as this EEG pattern was observable through the entire sleep episode (Figure 3). Temperature manipulations can induce alterations in the spindle frequency range (52). However, more in-depth analysis of this frequency range in WVD may provide a more distinct insight into the relationship between temperature changes and EEG development in this frequency range. The spindle pattern depends both on circadian phase (22) and a homeostatic component (21, 40).Therefore the complex interaction of circadian and homeostatic components regulating sleep together with circadian-, sleep-, and trait-dependent contributions to thermoregulation does not allow simple conclusions.

An apparently opposite thermal condition to WVD is described for patients suffering from narcolepsy (51). Narcoleptic patients show not only increased sleepiness, but also increased distal skin temperatures throughout the day when compared to CON (30). Polysomnographic recordings in narcoleptic patients revealed different NREM-REM sleep cycle lengths with an overall intact homeostatic sleep regulation (39).

Whether these observations are in turn linked to the reported temperature measurements needs to be directly measured in the same subjects.

All the described differences in WVD with respect to CON are inter-correlated, also with the observed circadian phase delay. None of them are established measures that could influence NREMS pressure. Circadian differences alone cannot explain all the findings (e.g. for REMS). But whether the increased heat retention of WVD around the sleep onset period has an impact on the following sleep pattern remains unclear, as no data exist from posture- and temperature-controlled studies with subjects having such a diathesis. Some of the effects of body heat load on sleep have been measured; however no study exists showing effects on sleep after a cold load, i.e. a study with provoked cold extremities limited to the sleep onset period. Ongoing analysis of our investigations into the influence of temperature manipulations by water immersions prior to a sleep episode on thermoregulatory and EEG patterns in WVD may provide more information in this regard.

In summary, WVD present sleep EEG patterns that have been reported in sleep following naps, insomniacs, depressed subjects, older individuals, DSPS patients, or the inverse findings in narcolepsy. WVD exhibit a variety of altered symptoms in different physiological and psychological areas when compared to women not having this diathesis, namely differences in thermoregulation, prolonged SOL, distinctive autonomic and psychological features and an unequal sleep EEG pattern.

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CHAPTER 4

BODY HEAT LOSS AND HEAT GAIN: IMPACT ON SLEEP IN WOMEN WITH VASCULAR DYSREGULATION AND DIFFICULTIES INITIATING SLEEP

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ABSTRACT

Study objectives

The study was designed to investigate whether any aspects of the sleep EEG can be modified by body heat loss or body heat gain in women with vascular dysregulation and difficulties initiating sleep (WVD).

Design

Using a shortened version of a controlled constant routine protocol, three baths at intervals of at least 4 days in a balanced randomized order were taken in the afternoon, followed by a 2-h nap.

Setting

University-based Chronobiology laboratory.

Participants

Nine healthy young women without any thermoregulatory or sleep complain (CON) and nine healthy young WVD.

Interventions

A 35-min afternoon head-out water immersion at three different temperatures (warm 39℃, neutral 35℃, and cool 28℃) followed by a su bsequent 2-h nap.

Measurements and Results

After a cool bath, WVD manifested a lower distal-proximal temperature gradient, a higher core body temperature during the nap, a faster rate of REM sleep accumulation with shorter REM sleep latency and lower SWA compared to CON. Sleep onset latencies were affected by the different temperature interventions only in CON, with shortest latencies after warm bathing. In general, WVD developed lower EEG power values than CON in the theta and alpha frequency range of the EEG power spectra.

Conclusions

Cool bathing affects sleep in women with relatively high body heat retention (WVD) stronger than in CON, most pronounced for REM sleep, and low frequency EEG power in the frontal cortex . In contrast, warm bathing in WVD vanishes most of these differences.

INTRODUCTION

Core body temperature (CBT) is a reliable and convenient physiological variable that has been used since decades as “the” marker of the human circadian sytem (65), and is strongly related to sleep regulation (63). The sleep-wake cycle is generally synchronized with the circadian rhythm of CBT: the crest of the circadian rhythm of sleep propensity (i.e. short sleep onset latency [SOL]) occurs during the declining portion of the CBT and its trough at the CBT maximum (22). Sleep architecture is also related to changes in CBT. A relationship has been reported between proximity of the maximum rate of decline of CBT to SOL and sleep efficiency measures, and the amount of slow wave sleep (SWS) accumulated during the sleep episode (51). Additionally SWS decreases if the usual decrease of CBT is attenuated for example by a high ambient temperature (29). There is a close temporal relationship between rapid eye movement sleep (REMS) and CBT: REMS latency (REML) is shortest, REMS episode duration longest, and the amount of REMS greatest around the circadian CBT minimum (18, 22). A positive and negative correlation between CBT and REML, and CBT and amount of REMS, respectively, has been reported after warm bathing and a hot footbath (59). A more rapid accumulation of REMS and a tendency towards increased REMS duration was observed after advancing the CBT nadir by manipulating ambient sleep temperature (20). Phase delaying CBT rhythm after manipulating ambient sleep temperature, increased its amplitude and increased accumulation of SWS at the expense of REMS accumulation (60).

Although CBT globally correlates with SOL, it is not the key factor. The decline in CBT is a consequence of heat loss via distal skin regions, not a primary cause of sleepiness induction (45). The best predictor for a short SOL is vasodilated distal skin regions (warm hands and feet) and peripheral heat loss (38, 40, 41, 43, 59). The concept that it is body heat loss which is crucial for sleepiness and sleep initiation, is supported by a number of studies (9, 28, 50, 62). The endogenous circadian rhythm of CBT which results from a balance between heat production and heat loss (3, 42) can be effectively manipulated by body immersion in warm or cold baths. Through such interventions, CBT changes directly by external uptake or liberation of heat via conductive heat transfer. Some of the effects of body heat load on sleep have been measured: passive heat load shortens SOL and increases SWS (11, 24, 25, 33, 36, 37, 59).

In a series of experiments we have been studying women with a primary vascular dysregulation (VD) under ambulatory and stringently controlled laboratory conditions. VD has been described as a functional vascular dysregulation in otherwise healthy subjects, whose main symptom is thermal discomfort from cold extremities (hands and feet) (26). Subjects with VD often suffer from difficulties initiating sleep (DIS) due to their cold extremities (44, 52). In other words, they can be characterized as having a disposition for impaired distal vasodilatation, hence restricted heat loss via heat redistribution from the core to the shell (2, 40). Individuals with VD and concomitant DIS provide therefore a model of nature to study the relationship between thermophysiology and sleep without any invasive manipulations.

No controlled study exists showing the effect of temperature manipulations (e.g. by passive or active body heat load) on sleep in the afternoon at a circadian phase of relative CBT stability (no surplus of body heat loss), and no controlled study has been carried out on the effects of a cold load on sleep and sleep EEG power density. The homeostatic adjustment after heat and cold loads resembles the thermoregulatory effects of the falling and rising limbs of the CBT in the evening and morning, respectively. Under controlled conditions it will be possible to show whether SOL, SWS and low frequency EEG power in young healthy good sleepers (controls, CON) are dependent on body heat loss (after warm bathing, CBT increase) or body heat gain (after cool bathing, CBT decrease (without shivering)) compared with a neutral bath (no change in CBT). The same protocol will be applied to women with VD and DIS (WVD) to compare their behavior regarding a temperature intervention prior to a sleep episode with CON. This should additionally give insight into the effects of heat load interventions in a "model" thermoregulatory disorder such as VD in terms of improvement of the concomitant DIS, providing therwith an additional aspect of therapeutic relevance.

We hypothesize that SOL and the amount of REMS will decrease, SWS, SWA, and REML increase after body heat loss (i.e. warm bathing) and vice versa after body heat gain (i.e. cool bathing).

METHODS

Subjects

Two groups of healthy young women (WVD N = 9, and CON N = 9) were recruited via poster advertisements at the University of Basel and via announcements on the internet. Their characteristics (mean ± SEM) were: WVD: 24 ± 1.04 y; 59 ± 2.0 kg; height, 168.3 ± 2.0 cm; body mass index (BMI), 20.8 ± 0.5 kg/m^2 ; CON 25.11±1.7 y; 58.3 ± 2.2 kg; 167.2 ± 2.5 cm; 20.8 ± 0.6 kg/m^2). Exclusion criteria were extreme morning or evening types (61), chronic or current major medical illness or injury, amenorrhea or irregular menstrual cycles, smoking, intake of over-the-counter or prescription medications (including oral contraceptives or other hormonal treatments) or illicit substances, shift work within 3 months or transmeridian travel within 1 month prior to the study, excessive caffeine (i.e. > 300mg) and alcohol consumption (i.e. > 1 beverage per day).

Subjects who fulfilled the above criteria were subjected to finger nailfold video capillary microscopy to objectively document their self-ratings about cold or warm extremities (inclusion criteria: WVD = blood standstill for ≥ 12 sec, CON = < 12 sec) (26, 27). Additionally nailfold skin temperature was measured. After a physical examination to exclude any medical disorders, a polysomnographically recorded screening night in the laboratory was performed to test their ability to sleep in a new environment, to exclude primary sleep disorders (such as insomnia) and to assess sleep onset latency to sleep stage 2 (≥ 20 min for WVD, < 15 min for CON, see Table 1). Subjects with sleep efficiency lower than 80% were excluded from participation.

All selected subjects entered the study after the fourteenth and before the first day of their menstrual cycle in order to complete the experiment within the luteal phase. During 3 days before admission to the laboratory (baseline days, BL) subjects were instructed to maintain a regular sleep-wake schedule (bedtimes and wake times within ± 60 min of self-selected target times scheduled 8 h apart). Adherence to this regular schedule was verified with a wrist activity monitor (Cambridge Neurotechnologies®, UK) and sleep-wake logs. They were also instructed to abstain from excessive caffeine and alcohol consumption (definition see above) as well as heavy physical exercise. The nature, purpose, and risks of the study were explained

before subjects gave their written consent. It was explicitly permitted to stop the experiment at any time. The study protocol was approved by the local ethical committee ('Ethikkommission beider Basel') and conformed to the guidelines contained within the Declaration of Helsinki. All 18 subjects completed the study without any complaints.

Study design and protocol

Subjects underwent three study blocks in a randomized balanced order with an interval of at least four days in between: Each block consisted of a shortened controlled constant routine protocol (CR) to exclude any masking effects on thermoregulation due to behavioral and environmental influences (for details see (64)), including a 35-min head-out water immersion followed by a 2-h sleep episode and 1 h CR. In order to achieve three different thermophysiological states prior to sleep baths at three different temperatures were performed (cool 28℃, neutral 35℃ and warm 39℃, respectively). To set the 2 h sleep episode during a stable circadian time point, the timing was individually calculated such that the afternoon sleep was set 9 h after usual wake-up time (obtained by centering an 8-h sleep episode at the midpoint of each volunteer's habitual sleep episode as assessed by actigraphy and sleep diaries during BL). Subjects reported to the laboratory 2 h before the CR which was set 12 h before habitual bedtime. They wore a two-piece bathing suit and a light cotton nightdress covering arms and knees. Electrodes for EEG and ECG measurements were attached while sitting. Thereafter subjects moved to the sound-attenuated chronobiology room (for details see (64)). After lying down, they were covered with a light blanket and were allowed to adjust their bedcovers to maintain thermal comfort. Fifty minutes after lying down subjects ate a light lunch (1200 kcal). Water was available ad libitum. 4.5 h after beginning the CR 35 minutes bathing intervention took place, i.e. 60 min before lights off. To avoid postural changes during the entire protocol, subjects were transported from the laboratory bed to the bath and back on a wheeled bed and hoisted into the bathtub by a crane already in hospital use.

Physiological measurements

Sleep recordings

Sleep episodes were polysomnographically recorded using the Vitaport Ambulatory system (Vitaport-3 digital recorder TEMEC® Instruments B.V., Kerkrade, The Netherlands). Six EEG derivations (frontal [Fz], central [Cz, C3, C4], parietal [Pz], occipital [Oz], referenced against linked mastoids, A1, A2), two electrooculograms, one submental electromyogram, and one electrocardiogram were recorded. All EEG signals were filtered at 30 Hz (fourth-order Bessel-type anti-aliasing low-pass filter, total 24 dB/octave), and a time constant of 1.0 second was used prior to online digitization (range 610 µV, 12 bit analog-to-digital converter, 0.15 µV/bit; storage sampling rate at 128 Hz for the EEG). The raw signals were stored online on a Flash RAM Card (Viking, Rancho Santa Margarita, Calif) and downloaded offline to a personal computer hard drive.

Thermometry

Temperature data were continuously recorded by a portable computerized system (Almemo® 2590-9, Instr.Type MA2590-9, Serial No. H03070235G, Software No. 5.78, Ahlborn, Holzkirchen, Germany) in 10-sec intervals (for details see (64)). Rectal temperature and skin temperatures from 8 sites (proximal: forehead, infraclavicular, stomach, thigh; distal: hands feet) were continuously measured (for details see (64)). Thereafter, temperature data and the distal-proximal skin temperature gradient (DPG, for details see (40)) were adjusted to lights off (=0) and binned into 5-min intervals for statistical analysis.

Data Analysis

EEG stage analysis

All sleep episodes were visually scored (Vitaport Paperless Sleep Scoring Software; TEMEC® Instruments) for consecutive 20-s epochs (C3-A2 derivation) according to standard criteria (32, 55). SOL was defined as the time interval between lights off and the occurrence of the first 20-sec sleep epoch of sleep stage 2. REM sleep latency (REML) was calculated from sleep onset (defined as latency to stage 2). All sleep latencies (latency to stage 1[SL1], latency to stage 2 [SL2], latency to stage 3 [SL3],

latency to stage 4 [SL4], REML) were log - transformed before statistical analysis. Total sleep time (TST) was defined as Stage 1 + 2 + 3 + 4 + REM sleep. Sleep efficiency (SE) was defined as follows: SE = TST/time between lights off and lights on x 100. Wakefulness after lights off (WALO; % of TST) was also calculated. Non-rapid eye movement sleep (NREMS) was defined as stages 2 to 4 (% of TST). Sleep stages (1-4), rapid eye movement sleep (REMS), wakefulness, and movement time (MT) were expressed as percentage of total sleep time (TST) during the respective night for all participants (Σ stages 1-4, REMS). TST and sleep latencies and REML are indicated in minutes. Time courses of cumulative sleep stage data were calculated for the first 90-minutes in 1-minute intervals after onset of sleep stage 2.

EEG spectral analysis

EEGs were subjected to spectral analysis using a fast Fourier transform (10% cosine 4-s window), resulting in a 0.25 Hz bin resolution. EEG artifacts were detected by an automated artifact detection algorithm (Vitascore, CASA; 2000 Phy Vision B.V., Kerkrade, The Netherlands). For final data reduction, the artifact-free 4-s epochs were averaged over 20-s epochs and matched with the 20-s epochs of the visual sleep scoring.

The effect of different bathing conditions (cool, neutral, warm) on EEG power density spectra of NREMS was calculated for the midline Fz and Oz derivation in the frequency range of 0.5-25 Hz. The evolution of EEG power in the low frequency range during the first 90 min after sleep onset is illustrated by cumulative EEG power values of the 1Hz bin (log transformed values) in 1-minute intervals. This frequency bin was chosen because it represents the maximum value of the power EEG power spectra. All data were log - transformed before statistical analysis.

Statistical analyses

The statistical packages StatView™ 5.0, SuperANOVA™ (Abacus Concepts, Berkeley, California, USA), and STATISTICA 6 ™ for Windows (StatSoft Inc., Tulsa, USA) were used. For statistical differences the threshold for alpha-errors was set at $P < 0.05$ (two-sided, not especially indicated).

Data analyses of temperatures were performed by three-way rANOVA with factors *group* (WVD, CON) *x bath* (cool, neutral, warm) *x time course* (30 x 5 min bins). Additionally, differences between CON and WVD were tested for each bathing condition separately using Student's unpaired *t* - test.

For the entire sleep episode, sleep stages, SL1, and SL2 were analyzed with data of all subjects by two-way ANOVA for repeated measures (rANOVA) with factors *group* (WVD, CON) *x bath* (cool, neutral, warm). Additionally this analysis was repeated at time 55 minutes after onset of sleep stage 2. This time was chosen because maximal discrimination between factors bathing conditions and group has been occurred. For REML, subjects who had no REMS were excluded (cool condition: CON=7 and WVD=8; neutral: 8 and 7; warm: 9 and 7). Additionally the difference between WVD and CON in the time courses of cumulative sleep stages was statistically tested by t-tests for each bathing condition separately.

In order to analyze reliable data for spectral analyses participants with a sleep efficiency of less than 68% (16.6% of all sleep episodes) were excluded; 1 WVD, 1 CON in cool condition, 3 WVD, 1 CON in neutral condition, 2 WVD, and 1 CON in warm condition; no statistical difference between WVD and CON was found in the number of excluded subjects). Therefore in order to increase statistical power statistical significances between CON and WVD were tested for each bathing condition separately. For cool, neutral and warm condition t-tests of power density data were performed for each frequency bin (0.5-25Hz) 55 minutes after onset of sleep stage 2. This time was chosen because there was maximal discrimination between factors bathing conditions and group (see above). At the same time, a three-way rANOVA was calculated for the 1 Hz bin with factors *group x bath x derivation* with complete data set for all bathing conditions (N=7 CON and 6 WVD). Additionally the difference between WVD and CON in the time course of cumulative EEG-power values of the 1 Hz bin was statistically tested by t-tests for each bathing condition separately.

All *P* values derived from rANOVAs were based on Huyhn-Feldt corrected degrees of freedom, but the original degrees of freedom are reported. For *post-hoc* comparisons Fisher's PLSD with alpha-correction for multiple comparisons according to Curran-Everett (17) were calculated. Means ± standard error of the mean (SEM) values are presented.

RESULTS

Temperature analysis

Time course of CBT and DPG before, during and after bathing

Temperature time courses after cool (28℃), neutral (35℃) and warm (39℃) bathing are shown in Figure 1 for 15 minutes before, 2 h during, and 15 minutes after sleep. A three-way rANOVA with the factors *group*, *bath, and time course* revealed for DPG a significant main effect *group* ($F_{1,16}$ = 6.56, P < 0.05) and significance for the interaction term *group* x *bath x time course* ($F_{58,928}$ = 3.27, P < 0.05). WVD exhibited generally lower DPG values than CON. Post hoc analyses showed after cool bathing significant differences 40 min after lights off. After warm bathing WVD showed lower DPG values during the 10 minutes before lights off. For CBT a three-way rANOVA with the factors *group*, *bath, and time course* revealed a significant interactionterm *group* x *time course* ($F_{29,464}$ = 2.21, P < 0.05) with WVD showing a generally damped decrease of CBT during the sleep episode after bathing. After cool bathing CBT of WVD tended towards significance starting 95 minutes after lights off until 10 minutes after lights on (P < 0.1) and reached even significance 10 minutes after lights on (P < 0.05). To further disclose differences regarding body heat distribution in WVD and CON, a rectal – distal skin temperature gradient (RDG) was calculated (data not shown). A three-way rANOVA with the factors *group*, *bath, and time course* revealed for RDG a significant main effect group ($F_{1,16}$ = 4.41, P = 0.05) with WVD showing generally a higher RDG than CON. Additionally, significance was reached for the interaction term *bath x group* ($F_{2,32}$ = 3.92, P < 0.05) and for the interaction term *bath x time x group* ($F_{58,928}$ = 4.29, P < 0.05). Post hoc comparison revealed that WVD had a higher RDG after the cool bathing condition than after neutral and warm bathing, respectively, compared to CON. The difference in RDG started 25 minutes after lights off until 15 minutes after lights on.

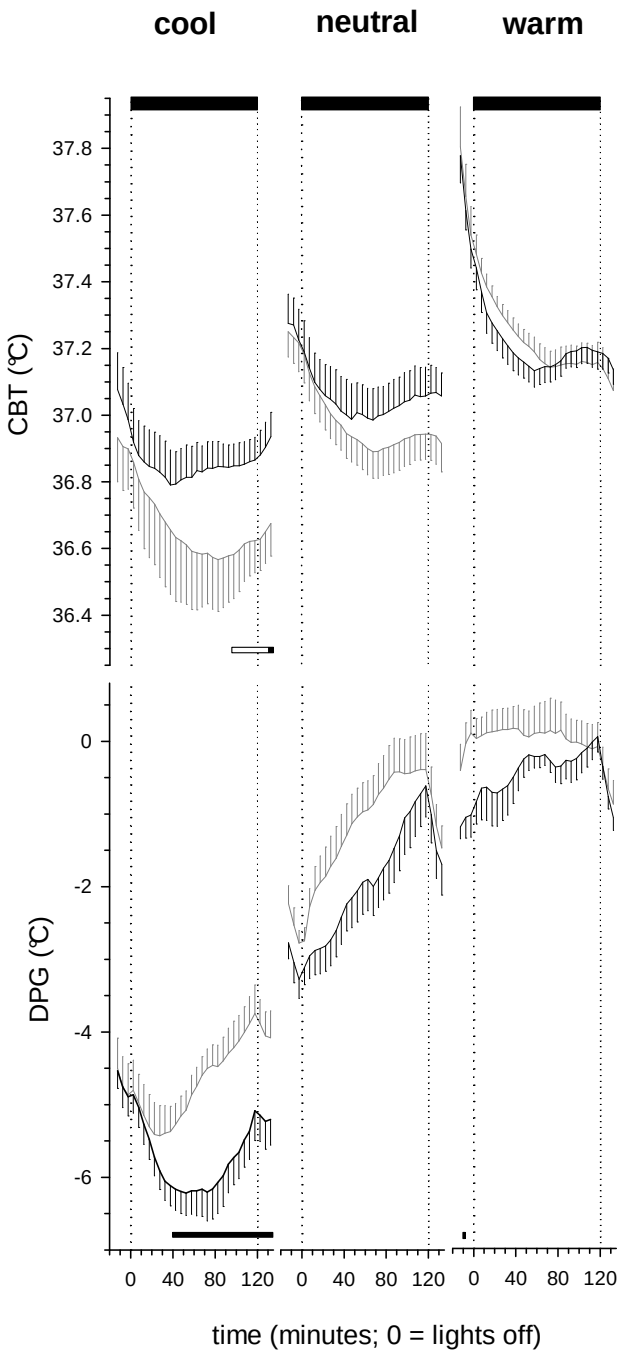

Figure 1. *Time course of core body temperature (CBT) and distal-proximal skin temperature gradient (DPG) before and during a 2-h nap after bathing at three different temperatures (28℃, 35℃, 39℃) for controls (CON, gray line, N=9) and women with vascular dysregulation and difficulties initiating sleep (WVD, black line, N=9). Data represent mean ± SEM. Black bars at the bottom indicate significant differences between WVD and CON (p<0.05); open bars indicate tendency (p<0.1); Dotted line indicates the time of lights off and lights on, respectively; Black bars at the top indicate sleep episode.*

Analyses of the EEG-signal recorded during the 2h-nap

Sleep stages

Inspection of Table 1 reveals no differences in any sleep stage between WVD vs. CON and between the three bathing conditions. Only REML exhibited a significant bathing effect as WVD had a shorter REML after cool and neutral bathing. Additionally, a two-way rANOVA for SL2 with the factors *group* (WVD vs CON) and *bath* (cool, neutral warm) revealed a tendency for the interaction term *group x bath* ($F_{1,2}$ = 2.90; P < 0.07). Post-hoc comparisons revealed longer SL2 in CON after cool than after warm bathing, whereas SL2 of WVD was not affected by different bathing

temperatures. For SL1 a tendency for the main effect *bath* was found ($F_{2,32}$ = 2.74; P < 0.1) with a longer SL1 after the cool bathing than after the neutral bathing. For percent of stage 1 a tendency for the main effect group was found ($F_{1, 2}$ = 3.50; P < 0.1) with CON having less stage 1 than WVD.

Table 1. Sleep measures derived from visual scoring of the 2-h nap after cool (28℃), neutral (35℃) and warm (39℃) bathing between CON (N=9) an d WVD.

Variable	CON cool	WVD cool	CON neutral	WVD neutral	CON warm	WVD warm
TST	97.3 ± 5.8	98.2 ± 6.8	102.9 ± 8.3	92.4 ± 10.4	103.3 ± 7.9	93.1 ± 9.0
SE %	80.8 ± 4.8	81.6 ± 5.6	85.4 ± 6.9	76.8 ±8.6	85.7 ± 6 .5	77.4 ± 7.5
WALO	21.5 ± 5.8	20.7 ± 6.8	16.3 ± 8.4	25.9 ± 10.0	15.4 ± 7.7	24.9 ± 8.8
MT	1.5 ± 0.4	1.4 ± 0.4	1.3 ± 0.3	2.1 ± 0.7	1.7 ± 0.3	2 .4 ± 0.5
arousal	31.0 ± 5.8	34.3 ± 7.5	26.5 ± 8.5	38.3 ± 9.4	24.3 ± 7.9	38.5 ± 8.9
stage 1	8.0 ± 1.1	12.2 ± 1.8	8.9 ± 0.8	10.3 ± 2.2	7.1 ± 1.1	11.3 ± 1.8
stage 2	50.3 ± 5.2	48.8 ± 5.3	47.2 ± 4.7	43.1 ± 4.8	51.7 ± 5.5	43.2 ± 3.7
SWS	30.2 ± 7.1	25.7 ± 4.7	34.9 ± 3.6	27.7 ± 4.4	31.5 ± 5.2	30.3 ± 5.8
NREM	80.4 ± 6.3	74.5 ± 5.6	82.1 ± 7.7	70.9 ± 7.1	83.2 ± 7.4	73.5 ± 7.4
REM	8.8 ± 2.4	11.4 ± 2.6	11.9 ± 2.9	11.2 ± 2.8	12.9 ± 3 .5	8.4 ± 2.3
SL1 (§)	11.0 ± 2.3	7.3 ± 1.3	5.4 ± 1.0	6.8 ± 1.9	5.7 ± 1.3	8.2 ± 2.1
SL2 (#)	14.0 ± 1.9	11.9 ± 1.5	9.4 ± 0.9	11.6 ± 2.0	8.8 ± 1. 8	13.3 ± 2.4
REML*	66.9 ± 8.5	53.2 ± 6.8	68.4 ± 7.0	60.1 ± 8.7	68.1 ± 8.7	62.7 ± 7.6
stage 1 % *(*)*	8.4 ± 1.1	13.6 ± 2.7	10.1 ± 2.4	11.1 ± 1.7	7.5 ± 1. 3	12.9 ± 2.3
stage 2 %	52.5 ± 4.7	49.4 ± 3.7	45.4 ± 1.8	49.2 ± 5.5	50.1 ± 3.3	48.2 ± 4.3
stage 3 %	11.4 ± 1.0	10.5 ± 1.2	15.4 ± 2.6	10.4 ± 3.4	12.6 ± 2.2	12.0 ± 2.5
stage 4 %	18.5 ± 6.0	15.8 ± 4.0	18.2 ± 2.0	19.0 ± 4.1	18.1 ± 4.4	19.0 ± 4.5
SWS %	29.9 ± 6.3	26.3 ± 4.0	33.6 ± 1.8	29.4 ± 4.5	30.7 ± 4.0	31.0 ± 4.0
NREM %	82.4 ± 2.9	75.7 ± 1.9	79.0 ± 2.6	78.7 ± 3.1	80.8 ± 3.3	79.3 ± 2.9
REM %	9.2 ± 2.4	10.7 ± 2.3	10.8 ± 2.8	10.3 ± 2.4	11.7 ± 3 .2	7.7 ± 2.0

*Values are in minutes or in percentage of total sleep time (TST = stage1 + 2 + 3 + 4 + REM sleep), SE = sleep efficiency [(TST/time in bed) x 100]. WALO = wakefulness after lights off, MT = movement time, arousal = WALO + MT + stage 1, SL1 = latency to stage 1 (min), SL2 = latency to stage 2 (min), SL3 = latency to stage 3 (min), SL4 = latency to stage 4 (min), REML = latency to REM sleep (min) calculated from latency to stage 2 to the first REM sleep episode. For SL1, SL2, SL3, SL4, and REML, statistics were applied on log-transformed values. (#) = p<0.07, interaction term: GROUP (WVD vs. CON) x bath (cool,neutral,warm); * = significant (p<0.05) main effect: GROUP. (*) = p<0.1. § = significant (p<0.05) main effect: BATH. (§) = p<0.1.*

Cumulative sleep stages after sleep onset

In order to demonstrate differences in the dynamics of sleep architecture during the first 90 min after sleep onset (to sleep stage 2) 1-min cumulative values of selected sleep stages were calculated and plotted for WVD and CON in all three bathing conditions (Figure 2). A three-way rANOVA with the factors *group*, *time* (18 x 5min intervals), and *bath* revealed a significant interaction term *group x time x bath* ($F_{34,544}$ = 1.73; P < 0.05). Post-hoc comparisons revealed a significant earlier accumulation of sleep stage REM after cool bathing in WVD than in CON. These differences in the

dynamics of sleep architecture with different bathing conditions were mainly induced by changes within WVD.

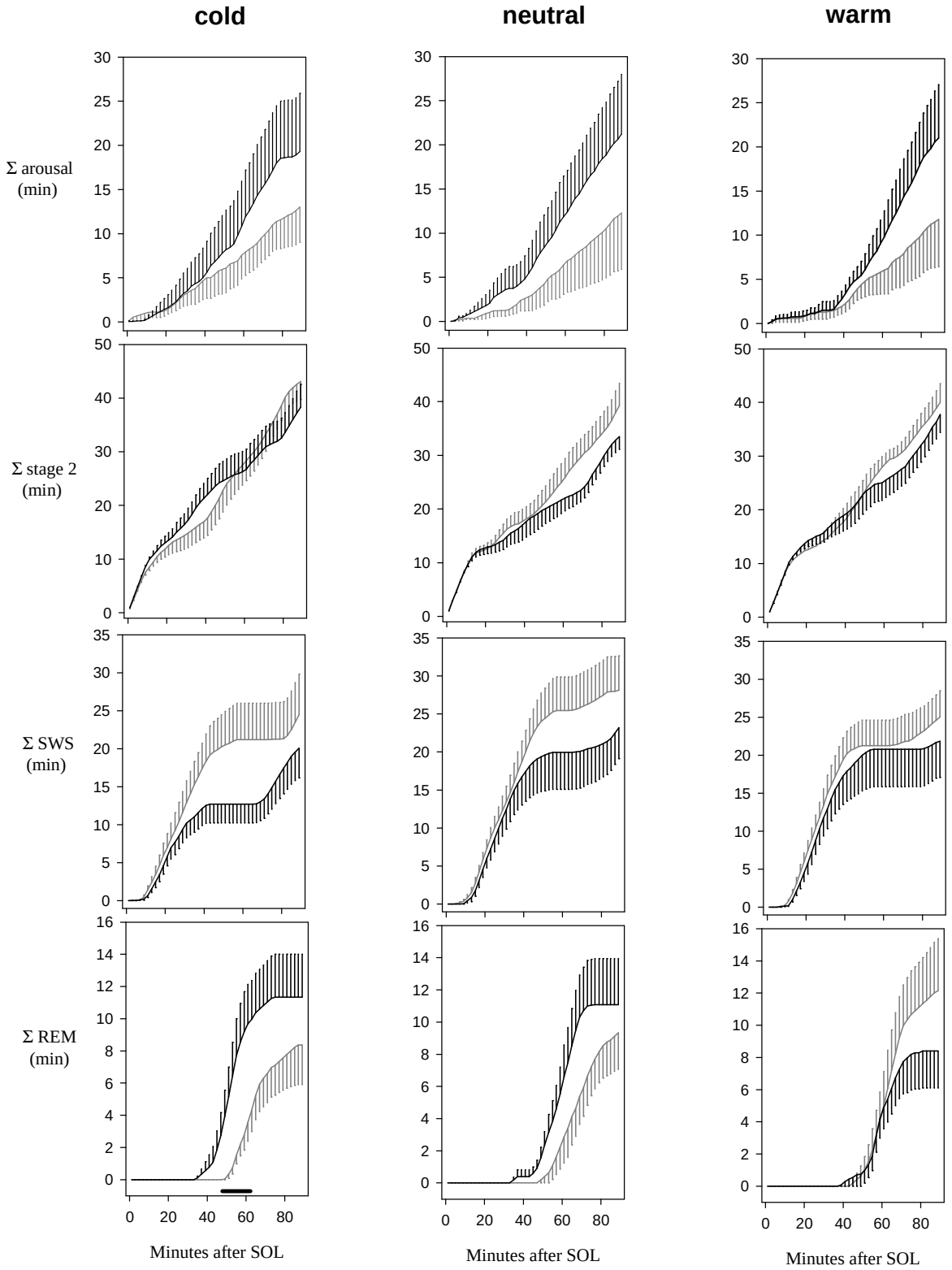

Figure 2. *Accumulation of sleep stages in 1-min intervals (grey lines: CON, N=9), black lines: WVD, N=9); mean values; vertical lines represent SEM). Black bars at the bottom of the panels indicate significant differences between WVD and CON (P<0.05).*

Changes in EEG power density (0.5 - 25Hz) after sleep onset

To describe the dynamic of sleep processes, EEG spectral analyses were performed. To examine EEG power density in the range of 0.5 - 25Hz during NREMS for CON and WVD with additional respect to topographical information, power density at the time point of 55 min after sleep onset was calculated for the midline derivation Fz and Oz and plotted as log transformed values for WVD and for CON of all three bathing conditions (Figure 3). A three-way rANOVA with the factors *derivation* (Fz, Oz), *bath*, and *group* revealed a tendency fort he interaction term *derivation x bath x group* ($F_{2,22}$ = 2.92, P < 0.1). Post-hoc comparison revealed that after cool bathing WVD exhibited significantly lower EEG power density in frequency bins of the delta, theta, and alpha frequency range (0.5 to 9.75 Hz; P < 0.05) in the Fz derivation, whereas in the Oz derivation lower power density was found only in some frequency bins of the theta and alpha frequency range (6.75 to 9.0 Hz; P < 0.05). After warm bathing, in both derivations Fz and Oz, WVD showed lower power density in some frequency bins of the theta frequency range (7.5 Hz to 8.5 Hz, and 6.0 Hz to 8.5 Hz, respectively; P < 0.05).

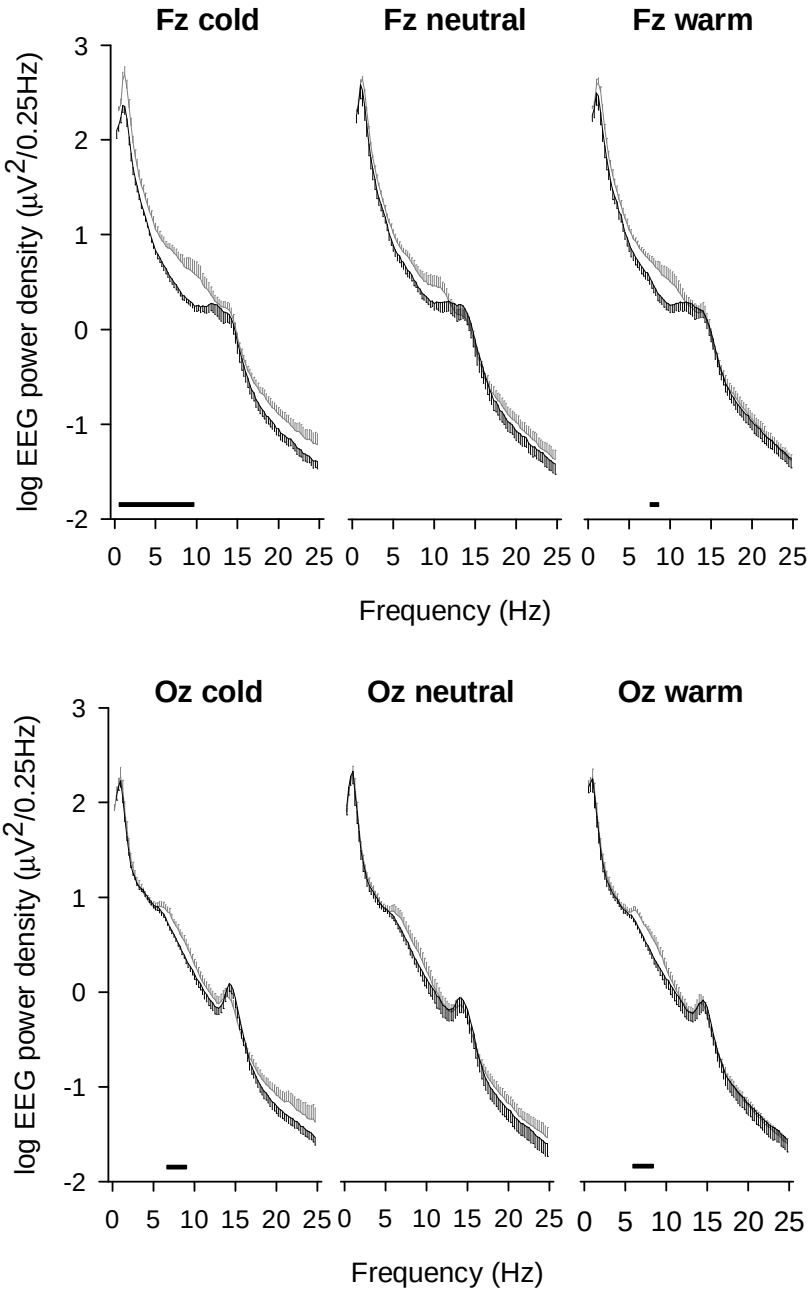

Figure 3. *Mean EEG power density during NREM sleep of the midline derivations Fz and Oz for the first 55 minutes after cool, neutral, and warm bathing. Mean +/- SEM are shown for each 0.25 Hz bin in the range of 0.5-25 Hz. Black bars at the bottom indicate significant differences between WVD and CON (P<0.05, number of subjects see METHODS).*

Cumulative power density after sleep onset

In order to demonstrate differences in the dynamic and in the temporal distribution of power density along the antero-posterior axis, in the low frequency domain during the first 90 min after sleep onset, 1-min cumulative power values of the 1 Hz frequency bin was calculated and plotted as log transformed values for WVD and for CON for the midline derivations Fz and Oz (Figure 4). After cool bathing, WVD showed lower EEG power than CON at the beginning (minutes 5 to 11; $P < 0.05$) and from the middle towards the end of the sleep episode (minutes 40 to 77; $P < 0.05$). Significant

lower EEG power of WVD after neutral bathing was observed at the beginning of the sleep episode (minute 16; $P < 0.05$). No differences between WVD and CON were found after warm bathing and in the Oz derivation.

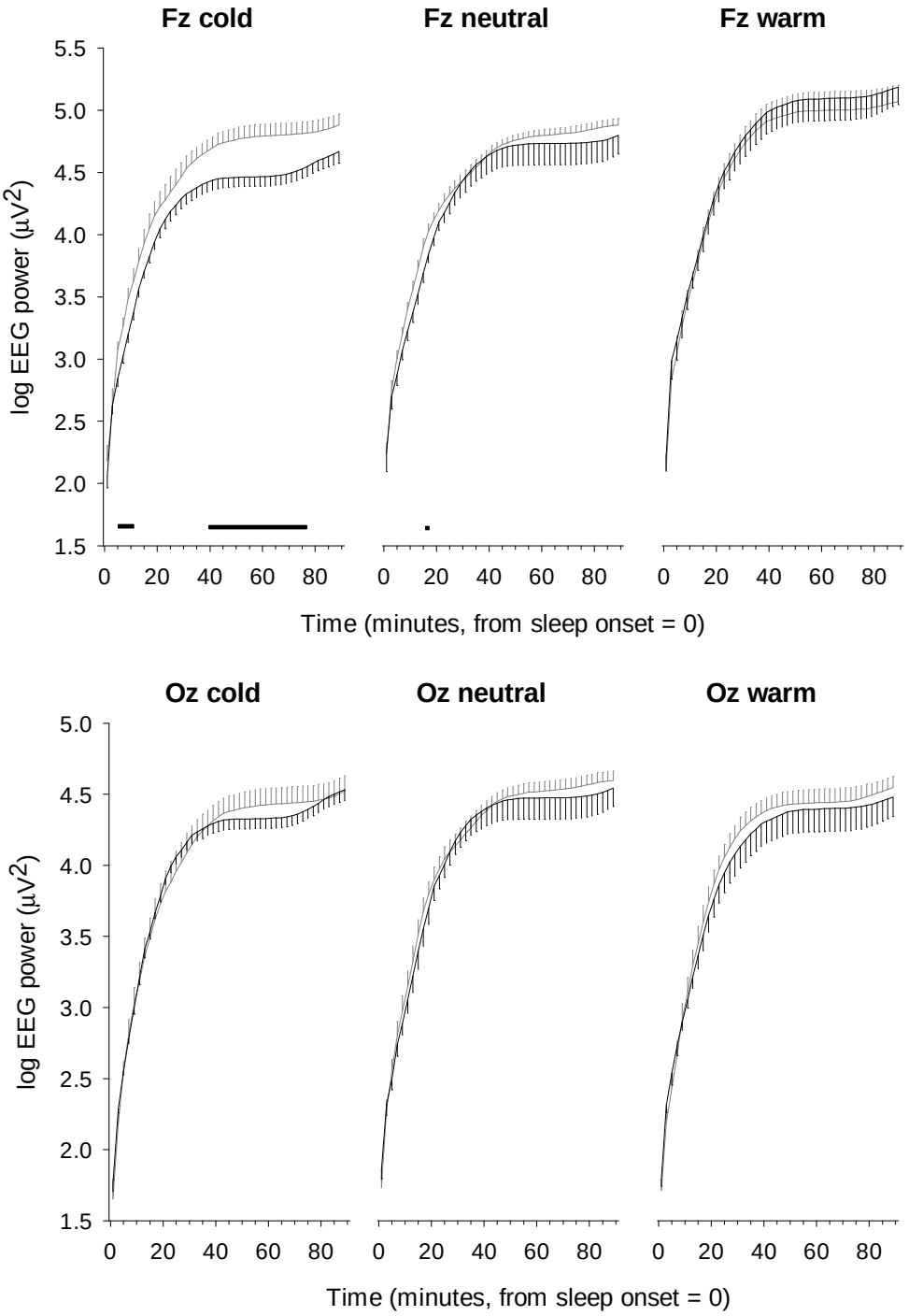

Figure 4. *Accumulation of EEG power of the 1Hz bin during NREM sleep of the midline derivation Fz and Oz for the first 90 min of the 2-h sleep in 1-min intervals (mean values; vertical lines represent SEM). Black bars at the bottom indicate significant differences between WVD and CON (P<0.05, number of subjects see METHODS).*

DISCUSSION

Warm and cool bathing induced two distinct thermoregulatory states before and during the subsequent afternoon sleep episode (Figure 1). Warm bathing induced elevated CBT and increased DPG in WVD and CON, which indicates a condition of thremoregulatory heat loss. Compared to CON, WVD responded to the relative cool ambient room temperature after the warm bath with a more pronounced distal vasoconstriction (lower DPG values) before lights off. Cool bathing induced reduced CBT and reduced DPG in WVD and CON indicating thermoregulatory body heat retention. The reduction in CBT and DPG occurred after bathing in cool water and not during the bath, which can be explained by heat redistribution from the core to the shell while the shell is re-warming after bathing, an effect that has been called 'afterdrop' (56). In WVD the core cooling was less pronounced than in CON, a consequence of the longer lasting distal vasoconstriction, i.e. the lower DPG values in WVD after the cool bath condition indicate a reduced heat redistribution from the core to the shell. This lower DPG was manifested 40 min after lights off until end of the 2-h nap and also during the following 15 minutes after lights on. WVD also exhibit a higher core to distal temperature gradient (RDG) than CON, starting 25 minutes after lights off and persisting throughout the 2-h nap. This additionally underpins the finding of an increased state of heat retention in WVD.

Sleep stage analysis revealed a shorter REML after cool and neutral bathing in WVD compared to CON. WVD showed after cool bathing an earlier and more rapid accumulation of REMS (Figure 2). As expected, SOL was longer after cool bathing than after warm bathing in CON. In contrast, no differences in SOL after the different temperature interventions were induced in WVD. During nocturnal sleep episodes WVD have longer baseline SOL (64). But during the afternoon naps they generally had shorter SOL. Other sleep parameters as SWS or REMS duration were not significantly affected by a warm or cold load in both, WVD and CON. Differences between WVD and CON became evident in the dynamics of sleep processes and with respect to the antero-posterior axis. WVD showed in general a lower EEG power density in frequency bins of the theta, and alpha frequency range irrespective of topography, i.e. frontal or occipital regions, or bathing condition. But in the delta frequency range WVD exhibited lower EEG power density after cool bathing in the

frontal region, a difference to CON that was no longer detectable in the occipital region and after warm bathing.

Small changes in core body, brain, or skin temperature can have a significant influence on EEG power spectra. For example, distal skin warming enhanced EEG power in the delta frequency range in frontal derivations (54). The simulation of the putative effect of brain temperature decrease on the human NREMS EEG revealed a decrease of EEG power density in the delta, theta and alpha frequency range (19), similar to findings in the human sleep literature (23). In WVD a decreased EEG power density was observed in the theta and alpha frequency range irrespective of a preceding thermal load. Therefore this finding may rather be trait than state dependent. WVD tended towards decreased EEG power density in the same frequencies already in a normal night's sleep (see Chapter 3). In contrast, EEG power density in the delta frequency ranges was dependent on the bathing condition in WVD as only after the cold load a decrease in EEG power density evolved, especially in the frontal regions. This dependency was not observed in CON, and was not expected, as others reported increased SWS - and presumably SWA - after warm loads. These findings are, however, limited to nocturnal sleep episodes and not afternoon sleep (36, 11). Compared to the cold load a warm load in WVD led to an increase in the delta frequency EEG power spectra until reaching the same level as CON which is in accordance with the literature (54). It has been reported and suggested that the amount of SWS is positively correlated with the magnitude of the decrease in CBT during sleep (11, 35, 58, 60) and not with the level of CBT as suggested by others (36, 57). A decrease of CBT is a consequence of increased heat loss via distal vasodilatation. The strong distal vasoconstriction in WVD after the cool bathing, that is a heat retention process, precludes the same pronounced decrease in CBT as observed in CON. This may therefore contribute to the lower delta EEG power density in WVD compared to CON. Regarding the amount of SWS, no differences with respect to the three different temperature interventions were found (Table 1). A finding that has been also observed in a study with elderly healthy subjects showing no alteration in the amount of SWS after a warm load inducing higher CBT throughout the following sleep episode (59). But inspection of Figure 2 points out to a more reduced accumulation of SWS in WVD after cool bathing than CON and this in turn would be in accordance with the literature (11, 35, 58, 60).

Is this pattern of SWS and SWA related to the observed shorter REML and steeper accumulation of REMS in WVD compared to CON after cool bathing?

REMS propensity is tightly coupled with the CBT rhythm. REML is shortest, REMS episode duration longest, and the amount of REMS greatest around the circadian CBT minimum (18, 22). Additionally a positive correlation between CBT and REML after warm bathing (59) and a more rapid accumulation of REMS after advancing the nadir of CBT (20) have been reported. Other reports of passive body heating provide contradictory results, on the one hand together with an increased CBT an increase of REMS (36) or a decrease of REMS after warm water immersion (34) or no effect on REMS at all (12, 35). Unfortunately, there is no information in these studies about distal skin temperature. And no studies are available reporting cool water immersion under controlled conditions with skin temperature and subsequent sleep recordings.

However, it may not be the level of CBT that impacts directly REMS, but the lower amount of SWA due to the strong vasoconstriction and impaired heat loss that favors the earlier occurrence of REMS in WVD after cool bathing. This is in accordance with observations from cooling down ambient room temperature during a sleep episode resulting in increased passive heat loss, lowered CBT, increased SWS accumulation at the expense of a similar REMS accumulation (60). Heat retention, i.e. a low DPG may be one manifestation of the physiological background, such as the hypothetical threshold that triggers stage REM (e.g. the Y* of McCarley (48) or the y^0 of Beersma (4)) at the expense of SWS and SWA.

A brain warming function of REMS has been suggested (66) which fits with findings of increased heart rate (15), peripheral vasoconstriction (47) and heat production during that sleep stage (8, 53). As a consequence, after a certain time lag small increases in CBT and skin temperatures have been documented following REMS (14, 31, 39). Because a low DPG normally results from a cool ambient temperature and is implemented to prevent body cooling, an additional early REMS onset may also prevent an impending CBT drop.

The enhanced heat retention process in WVD after cool bathing, together with the shorter REML and steeper REMS accumulation at the expense of SWS (Figure 2) may be an indication of an increased need for warmth in these subjects. In WVD the prevention of a CBT drop seems to have first priority over the manifestation of SWS. A condition of heat retention, i.e. reduced heat loss as a general trait of WVD, seems to favor REMS, because in nocturnal sleep under constant routine conditions WVD,

who are continuously in a heat retention mode, tended to have shorter REML (see Chapter 3) and a cool bath prior to an afternoon sleep episode may have accentuated this trait.

A difference in REML was only observed in WVD in relation to CON in the cool and neutral conditions. CON showed no differences in REML across the different temperature manipulations even though they exhibited a pronounced CBT drop during the sleep episode after cool bathing which, in accordance with the literature, should be in favor of REMS occurrence. For CON the temperature intervention may not have been strong enough to affect REMS. However, applying a stronger cold stimulus would have provoked a shivering response due to increasing metabolic rate which consequently would confound the outcome.

Another explanation of the earlier and steeper accumulation of REMS after a cold load in WVD compared to CON could also be found in subjective thermal perception of WVD. The imposed changes in peripheral temperatures may serve as a thermal signal operating at a central level and generating a psychophysiological stress in WVD who are particularly sensitive to cold, that in turn may induce REMS alterations (21).

Interestingly and at variance with our predictions, SOL in WVD was not affected by the different temperature interventions. Surprisingly, they exhibited in all three conditions a relatively low SOL when compared with their SOL values in previous nocturnal sleep episodes during a constant routine in the same chronobiological facility (see Chapter 3). There may have been a floor effect with respect to improvement especially for the warm bath condition as they already seemed to have reached their lowest possible SOL. CON in contrast showed similar short SOL after neutral and warm bathing to that observed in previous nocturnal sleep episodes but as expected a longer SOL after cool bathing. This longer SOL was probably due to the decreased DPG, as SOL is inversely correlated with DPG (41). However it was all the more surprising that in the cold-sensitive WVD SOL remained low even after the cold load. We chose a sleep episode in the afternoon at a circadian phase of relative CBT stability, i.e. at the high plateau part of circadian CBT rhythm. Regarding SOL, this time span of the CBT rhythm coincides with even longer SOL than observed on the falling limb of CBT rhythm during the night (22), but not with shorter SOL. And SOL of healthy young subjects in an afternoon nap has been reported to be similar to that in baseline nights (67) as was the case for CON in our study (see Chapter 3). The reasons for the general improvement of SOL in WVD in an afternoon nap even

after a cold load remains unclear, particularly as it has been reported that SOL of sleep onset insomniacs in the afternoon remains as expected on the basis of their nighttime sleep (30).

How can we explain the reduced EEG power density values in WVD not only regarding SWA but also regarding the theta and alpha frequency ranges?

The level of SWA is a robust measure of NREMS intensity and serves as an objective physiological indicator of sleep homeostasis (7), i.e. a high sleep pressure after sleep deprivation increases SWA most in the frontal region. Theta activity increases not only in the frontal but also in the occipital derivation (13). Therefore it could be assumed that WVD normally live under a reduced sleep pressure, as theta activity was reduced in the frontal and occipital derivation also after the warm bathing condition. Cool bathing reduced sleep pressure in WVD even more, leading additionally to reduced EEG power density in the delta range. That SWA may be altered during an afternoon nap due to a different circadian phase compared to a night sleep episode seems not to be an explanation as SWA values of CON in this afternoon nap was comparable with their values observed during a night sleep episode (data not shown). Similar findings have been reported for an afternoon nap compared with a night sleep in healthy young volunteers (67).

A significantly decreased level of alpha power and a deficit in delta and theta power during NREMS has been reported in insomniac patients (46, 49). This generally reduced slow frequency EEG activity during NREMS has been suggested to reflect a state of CNS hyperarousal (16). This may also apply to WVD. Additional evidence comes from the finding that WVD exhibit a predominance of sympathetic nerve activity (1) similar to insomnia patients (6). However, this suggestion of hyperarousal has its limitations as WVD exhibited a shorter SOL in the afternoon nap than in a night sleep episode. It is known that SOL is a function of the level of arousal (5) and therefore WVD should have rather shown longer SOL than CON, were this parallel true, in addition to the findings of the EEG spectral analysis.

Changes in sleep architecture could just be a result of an unspecific stress (10, 29) due to a thermal load. This is rather not the case in this study, as the salivary cortisol levels did not differ between WVD and CON during the protocol (data not shown).

In summary, this is the first study performed under strictly controlled constant routine conditions, showing the effect of a moderate cool and warm bath prior to a sleep episode on sleep parameters in subjects with a higher sensitivity towards cold stimuli

and with difficulties initiating sleep due to their cold extremities. It could be confirmed in good sleepers without vascular dysregulation (CON) that bathing at different temperatures (28℃, 35℃, and 39℃) in the afternoo n decreases and increases convective body heat loss via the distal skin regions, prolonging and shortening SOL in a subsequent sleep episode, respectively, however without affecting REMS, SWS, SWA, and REML. As the dynamics of CBT in CON were the same in all three bathing conditions but the level of CBT higher after the thermal warm load, it could be suggested that it is the dynamics of the CBT decline and not the level of CBT that impacts subsequent sleep (e.g. SWS, SWA). In WVD, cool bathing accentuated their already inherent state of heat retention and generated a shorter REML and a faster REMS accumulation compared to CON. This may be due to the lower amount of SWA and SWS accumulation as a consequence of enhanced heat retention, additionally indicating that heat loss mechanisms seem to be the determinant of SWS and SWA, respectively, and not directly the level of CBT. The lower log-EEG power density in the theta and low alpha frequency range in WVD seemed to be a trait-dependent feature, whereas the lower values in the delta frequency range appeared to be state-dependent.

However, the analysis of the temperature course after heat load and heat gain does not simply mirror the variations of EEG parameters. This finding indicates rather a complex relationship between body temperatures and alterations in the EEG power density spectra. It seems that it is rather the process and dynamics of thermoregulatory adjustment mechanisms that impact EEG parameters, than absolute values of CBT or skin temperatures.

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CHAPTER 5

CONCLUDING REMARKS

The main aim addressed by the experiments reported in the preceding chapters was to explain the relationship between thermophysiology and sleep by means of a 'model of nature' representing both, thermoregulatory and sleep related alterations. Individuals with a vascular dysregulation (i.e. disproportional intense response to a cold stimulus) and, due to this diathesis, difficulties initiating sleep ideally serve as such a model. Additionally, insight into this relationship should provide possible remedies to relieve the daytime and nocturnal distress of such affected persons.

Comparing chronobiological characteristics of WVD with CON during a CR in the first study (Chapter 2) revealed a difference in internal phase of entrainment of the thermoregulatory system relative to the sleep-wake cycle (a delay of the endogenous circadian rhythm in WVD). This was suggested to be the cause of their difficulties initiating sleep since DPG was lower in the evening before sleep onset, SOL was prolonged. The cause of this delay remains theoretical. It is known that temperature can impact the endogenous circadian pacemaker (see Introduction) but whether the observed daily cooler distal skin temperatures and slightly higher CBT in WVD compared to CON under both constant routine and daily life conditions is sufficient to induce phase shifts is unknown. There is also no information about whether a longer SOL may impact the circadian clock. The finding of a phase delay of CBT in sleep onset insomniacs (23) is not a proof for such causality, as no information about skin temperatures in those insomniacs was reported, and therefore some individuals in this group may have had VD. It remains to be dissected out whether the vascular dysregulation or the prolonged SOL alone induces the observed differences between WVD and CON.

That a different behavioral component could induce this phase delay via different exposure to Zeitgebers (e.g. light) can be ruled out, as WVD showed the same habitual bed- and wake-times as did CON. Another putative explanation is that WVD may have an abnormal phase response curves to light (PRC) in which the phase advance portion is weaker than normal. This has been suggested as a cause of delayed sleep phase syndrome (31). Delayed sleep may result from a reduced

sensitivity to one or more of the normal environmental entraining stimuli or a reduced phase advancing effect of correctly timed exposure to these entraining stimuli because of a possible alteration in internal coupling processes. Performing a study to generate a PRC would, however, be a somewhat disproportionate effort. A more feasible approach to test the phase shifting capacity of WVD is underway in the laboratory, by giving morning light pulse in order to advance the circadian rhythm of the endogenous pacemaker. Whether this would also work with a temperature pulse remains to be tested, because there are mostly only uncontrolled studies showing that changes in environmental temperature can act as a zeitgeber in humans (6, 7). Early experiments carried out under temporal isolation have shown that the free-running period shortens when ambient temperature during sleep is decreased by 6℃ (33) (p.95). This indicates that reduced temperature during the sleep phase induces a phase advance, and conversely, that elevated temperature induces a phase delay, which would suggest that temperature pulses have zeitgeber properties also for the human circadian system.

The sleep deprivation (SD) performed within the scope of a CR in the first study (Chapter 2) provided a stimulus of enhanced sleep pressure. As already reported in previous CR studies (18), the thermoregulatory system seems to be independent of the sleep homeostat. Here too, the SD stimulus had no effect on the thermoregulatory system in either WVD or CON. In terms of the sleep EEG, the SD stimulus initiated the well known effects on recovery sleep in both groups: shortened SOL and increased SWS and SWA (3, 4, 8) when compared to baseline sleep (Chapter 3). However, beside the prolonged SOL in WVD, sleep stage and sleep EEG power spectral analysis revealed some more general differences between WVD and CON: reduced duration of the first NREM-REM sleep cycle because of a diminished first NREMS episode, and a different evolution of delta power density across successive NREM-REM sleep cycles (the decrease in delta-power was less pronounced from the first to the second cycle) (Chapter 3). Based on similar sleep patterns found in nap studies (8, 10, 11, 32), in chronic insomniacs (19, 22) and depression (19, 20, 27), this observation is interpreted as a result of decreased NREMS pressure. The reasons in turn for this decreased sleep pressure remains theoretical. Practically any of the differences observed between CON and WVD could be responsible: the dominance of sympathetic activity in WVD indicated by a reduced HRV (1) and psychological features (30) may produce a condition of hyperarousal as

reported in insomniacs (2, 24, 25, 28) with concomitant lower EEG power in the delta, theta, and alpha frequency range (5, 9). The prolonged SOL of WVD per se seems to affect the following sleep pattern as it has been reported in exclusively sleep onset insomniacs (12). And whether the altered phase angle of WVD is responsible for the observed differences in sleep architecture remains to be tested by delaying their bedtimes by 1 h or advancing their endogenous circadian rhythm by 1 h. A close relationship between temperature and the first NREM-REM sleep cycle could be confirmed in this study, as DPG correlates positively with duration of the first sleep cycle. A different thermoregulatory pattern around sleep onset, i.e. reduced heat loss at the beginning of sleep, may impact on the sleep EEG not only in the first part of the sleep episode but also later on. For example the observed differences in EEG power density in the sigma frequency range, where WVD showed higher relative values in the spindle frequency range than CON, seems not to be directly (i.e. simultaneously) coupled to thermoregulatory processes as this EEG pattern was observable through the entire sleep episode. Temperature manipulations can induce alterations in the spindle frequency range (26). However, more in-depth analysis of this frequency range in WVD may provide a more distinct insight into the relationship between temperature changes and EEG in this frequency range.

A close relationship between thermoregulation and sleep has been suggested (see Introduction). The application of three different temperature stimuli in the second study (Chapter 4) resulted in different effects on thermoregulation and sleep: warm bathing (39°C) increased CBT in both WVD and CON with a high temperature persisting into the sleep episode, whereas a cold load (28°C) led to a remarkable decrease after bathing and during subsequent sleep. However, due to the more increased vasoconstriction of WVD this CBT decrease was less pronounced. The sleep stage and EEG spectral analysis revealed an interesting pattern: in WVD, cool bathing accentuated their already inherent state of heat retention and generated a shorter REML and a faster REMS accumulation compared to CON. This may have been due to the additionally observed lower amount of SWA and SWS accumulation as a consequence of the increased heat retention and impaired heat loss, respectively, which may constitute a determinant of SWS and SWA. The lower log-EEG power density in the theta and low alpha frequency range in WVD after cool bathing seemed to be a trait-dependent feature as it was virtually not affected by the temperature stimuli. In contrast, the lower values in the delta frequency range

appeared to be state-dependent, as they vanished after warm bathing. Cool bathing, hence reinforced heat retention in WVD, accentuated the sleep pattern already found under basal conditions (Chapter 2), as it further reduced EEG power density in the delta frequency range and shortened REML. These differences compared to CON seem to be influenced by the different thermophysiological state of WVD under normal life conditions, whereas the differences in the theta and alpha frequency ranges seem to be more related to the suggested higher activity of the sympathetic nervous system in WVD. Therefore, to test the latter suggestion experiments aiming at reducing sympathetic activity should be performed such as relaxation techniques, like suggestion of warmth (15), autogenic (13), or biofeedback training (13, 21). The impact of such techniques on the sleep EEG power spectra in WVD would provide more information on the cause of the observed EEG alterations that have been suggested to be trait-dependent.

However, analysis of the temperature course after heat load and heat gain does not simply mirror the variations of EEG parameters. Inconsistent observations are, for example, the low SOL of WVD even after cool bathing and despite their pronounced low distal skin temperatures, which is not consistent with the literature (16, 17). In contrast, CON showed the expected prolonged SOL after cool bathing. This is most likely due to the decreased DPG compared to warm bathing, as SOL is inversely correlated with DPG (17). Yet, apart from SOL, the temperature stimuli exerted no remarkable influence on the following sleep pattern in CON despite the induced different temperature states (low levels of CBT and DPG after cool and high levels after warm bathing). It seems therefore that it is not the level of e.g. CBT that impacts sleep architecture, because otherwise remarkable differences should have been found in the sleep patterns of CON. As the dynamics of CBT in CON were the same in all three bathing conditions (i.e. vasodilatation and subsequent decrease in CBT after lights off) but the level of CBT was higher after the thermal warm load, it could be suggested that it is the dynamics of the CBT decline and not the level of CBT that impacts subsequent sleep (e.g. SWS, SWA). This dynamic was altered in WVD.

In summary, WVD exhibit a variety of altered symptoms in different physiological and psychological areas when compared to women not having this diathesis, namely

differences in thermoregulation, prolonged SOL, distinctive autonomic and psychological features and an altered sleep EEG pattern. The findings of the two studies indicate a rather complex relationship between body temperatures and alterations in the sleep EEG. Future studies investigating WVD should also account for possible effects of other variables (e.g. hormones, neurotransmitters) that are known to influence sleep architecture (14, 29) and which might have additionally contributed to our complex findings.

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