Challenging the sleep homeostat does not influence the thermoregulatory system in men: evidence from a nap vs. sleep deprivation study

Kurt Kräuchi, Vera Knoblauch, Anna Wirz-Justice and Christian Cajochen
Centre for Chronobiology, University Psychiatric Clinics, Wilhelm Klein Strasse 27, CH-4025 Basel, Switzerland

Submitted to:
American Journal of Physiology (Regulatory, Integrative and Comparative Physiology)
Revised version: R-00381-2005 final accepted version

Running head: Thermophysiological correlates of sleepiness

Key words: circadian rhythm, sleepiness, sleep deprivation, skin and core body temperature, EEG Slow Wave Activity

Corresponding author:
Kurt Kräuchi
University Psychiatric Clinics
Centre for Chronobiology
Wilhelm Klein Strasse 27
CH-4025 Basel, Switzerland
Tel: + 41 61 325 55 08
Fax: + 41 61 325 55 77
kurt.kraeuchi@upkbs.ch

Copyright © 2005 by the American Physiological Society.
ABSTRACT

The purpose of our study was to understand the relationship between the components of the three-process model of sleepiness regulation (homeostatic, circadian, and sleep inertia) and the thermoregulatory system. This was achieved by comparing the impact of a 40h sleep deprivation versus a 40h multiple nap paradigm (10 cycles with 150/75min -wakefulness/sleep episodes) on distal (DIST) and proximal (PROX) skin temperatures, core body temperature (CBT), melatonin secretion (MEL), subjective sleepiness and nocturnal sleep EEG Slow-Wave Activity (SWA) in 8 healthy young men in a ‘controlled posture’ protocol.

The main finding of the study is that accumulation of sleep pressure increased subjective sleepiness and SWA during the succeeding recovery night, but did not influence the thermoregulatory system as measured by CBT, DIST and PROX.

The circadian rhythm of sleepiness (and PROX) was significantly correlated and phase locked with CBT, whereas DIST and MEL were phase advanced (by 113±28min and 130±30min, respectively; both p<0.005). This provides evidence for a primary role of distal vasodilatation in the circadian regulation of CBT and its relationship with sleepiness.

Specific thermoregulatory changes occur at lights off and on. After lights off, skin temperatures increased, most pronounced in DIST; after lights on, the converse occurred. The decay in DIST (vasoconstriction) was significantly correlated with the disappearance of sleep inertia. These effects showed minor and non-significant circadian modulation.

In summary, the thermoregulatory system seems to be independent of the sleep homeostat, but the circadian modulation of sleepiness and sleep inertia is clearly associated with thermoregulatory changes.

INTRODUCTION

Sleepiness can be defined as a physiological need for sleep with a behavioral measure of the subject’s tendency to fall asleep at a certain time (sleep propensity) (3, 25). Sleepiness, and its converse ‘alertness’ (in the following only ‘sleepiness’ is used), is a regulated and important determinant of psychomotor vigilance and performance (19). Three major processes are considered to be involved in regulating sleepiness: 1. A homeostatic process that is manifested in a growing increase of sleep
propensity during the course of wakefulness that is dissipated during sleep (7, 14) 2. A circadian process controlled by the circadian pacemaker in the suprachiasmatic nuclei (SCN) that produces a maximum drive for alertness during the subjective day and a maximum drive for sleepiness during the subjective night (7, 14) and 3. A process of ‘sleep inertia’, which describes the phenomenon of low vigilance upon awakening even though sleepiness should be lowest at the end of a sleep episode (24). These three processes represent the compartments of the mathematical three-process-model for sleepiness (2, 18).

Many studies have shown that the level of EEG (electroencephalogram) slow-wave activity (SWA) is a robust measure of non-REM sleep intensity and may serve as an objective physiological indicator of sleep homeostasis (6). A close relationship between the duration of prior waking and SWA in the following sleep episodes has been demonstrated and mathematically described (1). A sleep deficit elicits a compensatory response of increased SWA - excess sleep has the opposite effect. This regulatory mechanism is referred to as sleep homeostasis.

An elegant way to assess the contribution of homeostatic and circadian components of sleepiness and sleep-EEG measures is by scheduling subjects to non-24 h sleep-wake cycles (days much longer or shorter than 24h, e.g. 28h, 20h, outside the range of entrainment) (16, 17). In these so-called ‘forced desynchrony’ protocols sleep occurs at many different circadian phases, while the sleep homeostat can be assumed to be in a near steady state.

The phase and amplitude of the circadian pacemaker cannot be measured directly. However, the circadian rhythm of core body temperature (CBT) is considered a good indicator, when measured under the controlled conditions of a ‘constant routine’ protocol (CR; 41). In normal life, CBT is differentially “masked” by various behaviors (e.g. food and fluid intake, motor activity, muscular exertion, postural changes), as well as external conditions (e.g. light, sound, room temperature, humidity). The CR was developed to reduce such masking effects and serves therefore as an excellent method to measure circadian aspects of thermoregulation. In order to exclude the effects of sleep on the CBT, sleep is not allowed during the CR. Therefore, with increasing duration of sleep deprivation (e.g. for 40h) the sleep homeostat is challenged and sleep pressure increases, which itself induces changes in other output variables.
A general relationship between thermoregulation and sleep regulation has been long hypothesized, e.g. sleep as an energy conservatory state (23, 49). In humans, sleep is typically initiated on the declining portion of the CBT curve when its rate of change is maximal (11, 21, 38, 50, 51). We have shown that body heat loss via distal skin regions (measured by the distal-proximal skin temperature gradient, DPG) is the variable most closely linked to subjective sleepiness and predicts sleep onset latency (30, 31, ). More recently, we have also shown that distal vasoconstriction after a nap (between 4 and 8 pm) or after night sleep (between 11pm and 7am) is correlated with disappearance of sleep inertia after lights on (34). Conversely, the evidence for a thermoregulatory role of sleep in humans is surprisingly weak. Some studies have demonstrated that sleep propensity can be modulated by circadian and behavior-induced changes in cutaneous temperature (for review see 47). However, most studies showing correlations of CBT decline with slow wave sleep (SWS) have not been carried out under controlled conditions, particularly posture: subjects usually lie down just before lights off (16, 43). Although this may appear a minor detail, for thermoregulation it is not. Such a change in body position alone decreases CBT and increases skin temperatures for at least 2 hours thereafter (27). This masking phenomenon has been entirely neglected in interpreting prior data on thermoregulation and sleep. Thus, for understanding the relationship between the thermoregulatory system and sleepiness (sleep) regulation, studies under controlled unmasking conditions before, during and after sleep episodes are needed.

The aim of our study was to understand the relationship between the components of the three-process model of sleepiness regulation (2, 18) and the thermoregulatory system. Therefore, in a 40h crossover study under constant posture conditions, we attempted to combine the advantages of the CR (unmasking conditions for the circadian CBT rhythm) with the forced desynchrony protocol in a very much shortened form (10 cycles with 150min of scheduled wakefulness and 75min of scheduled sleep episodes), allowing separation (for discussion of this expression see below) of homeostatic and circadian aspects. The nap protocol furthermore allows a systematic comparison of sleep and sleep inertia on thermoregulation at different circadian phases. Finally, a comparison of 8h sleep episodes before and following the two protocols further allows for an evaluation of the effect of high vs. low sleep pressure on the thermoregulatory system in relation to SWA decay kinetics.
METHODS

Subjects

Subjects were recruited via poster advertisements at the University of Basel. After successfully completing a brief telephone screening, they received detailed information on the study and 3 questionnaires: a morning-evening-type questionnaire (46), the Pittsburgh Sleep Quality Index (PSQI, 8), and an extensive questionnaire covering sleep habits, sleep quality, life habits, physical health and medical history. Subjects with self-reported sleep complaints (PSQI score ≥5) as well as extreme morning or evening types (score <12 or >23) were excluded from participation. Other exclusion criteria were chronic or current major medical illness or injury, smoking, medication or drug consumption, shift work within three months or transmeridian travel within one month prior to the study, excessive caffeine consumption and excessive physical activity. Subjects who did not fulfil any of the above exclusion criteria were invited to the laboratory and interviewed. A physical examination excluded medical disorders. They spent an adaptation night in the laboratory to test the ability to sleep in a new environment and to exclude primary sleep disorders (e.g. insomnia, apnea). All subjects gave signed informed consent. The local Ethical Committee approved the study protocol, screening questionnaires and consent form. All procedures conformed with the Declaration of Helsinki.

Sixteen healthy young subjects (8 men and 8 women) completed the study. Based on the influence of the menstrual cycle and contraceptives on the thermoregulatory system, only results of the eight men (age: range 21-29 years, mean 25.1 ± 1.0 s.e.m.; BMI: range 19.60 -24.69 kg/m², mean 21.90 ± 0.46) are presented. The entire data set was used for analyses of neuro-cognitive functions and sleep (9, 10, 26, 42). During the week preceding the study, subjects were instructed to maintain a regular sleep-wake schedule (bed- and wake times within ± 30min of self-selected target time, verified by sleep logs and a wrist activity monitor; Cambridge Neurotechnology™). They were also instructed to refrain from excessive physical activity, caffeine and alcohol consumption. Drug-free status was verified upon admission via urine toxicologic analysis (Drug-Screen Card Multi-6 for amphetamines,
benzodiazepines, cocaine, methadone, opiates and THC; von Minden GmbH, Germany). All subjects completed the study without any complaints.

**Design (see Figure 1)**

Subjects underwent two study blocks in a balanced crossover design: a sleep-deprivation (SD, constant dim light, <8 lux) and a nap protocol [NP; 10 alternating sleep-wake cycles (or nap cycles, NAP# 1-10) of 150min of scheduled wakefulness (light phase, <8 lux) and of 75min of scheduled sleep (dark phase, 0 lux), for details see 9, 26)]. The low light intensity (<8 lux) was chosen because it is below the threshold for suppressing melatonin secretion. Subjects reported to the laboratory in the evening for an 8h sleep adaptation episode. The timing of the sleep-wake schedule was calculated in such a way that the sleep episode was centred at the midpoint of each subject’s habitual sleep episode as assessed by actigraphy during the baseline week. On the next afternoon electrodes and thermocouples were attached. After a second 8h sleep episode at habitual bedtime (baseline sleep), subjects remained in bed for 40h (semi-recumbent during wakefulness and supine during scheduled sleep episodes) under controlled constant routine (CR) conditions (room temperature 22°C, humidity 60%, light bedcover, 100kcal sandwiches, and 100ml water at 1h intervals; for details see 28). They either remained awake for a 40h total sleep deprivation or they completed 10 nap cycles. The protocol ended with a third 8h sleep episode (recovery sleep) starting again at habitual bedtime. After a 1-4 week interval, the subjects carried out their second study block.

**Thermometry**

Temperature data were continuously recorded by thermocouples (Interstar, Cham, Switzerland) in 20-sec intervals using a computerized system (System Hofstetter, SHS Allschwil, Switzerland). Core body temperature (CBT) was measured with a probe inserted 10 cm into the rectum. Skin temperature probes were fixed with thin air-permeable adhesive surgical tape (Fixomull™, Beiersdorf, Hamburg, Germany). Distal temperatures (DIST) were measured on the center of the back of the left and right hand, and the middle of the left and right foot instep (all later averaged). Proximal temperatures
Thermophysiological correlates of sleepiness

(PROX) were defined as the weighted mean of midforehead, weighting factor $f=0.093$; midthigh on the right musculus rectus femoris, $f=0.347$; right infraclavicular area, $f=0.266$; and stomach, 1cm above the navel, $f=0.294$. We omitted to present the distal-proximal skin temperature gradient (DPG; 30) because it would extend too much our Result-section (see comments below).

Salivary melatonin

Saliva samples were collected at half-hourly intervals for measurement of melatonin (MEL) using a highly specific direct double-antibody RIA (48).

Subjective sleepiness ratings

During the wake episodes subjective sleepiness was self-assessed at half-hourly intervals on the Karolinska Sleepiness Scale (KSS; 3).

Sleep recording and analysis

Sleep was recorded polysomnographically using the VITAPOR™ digital ambulatory sleep recorder (Vitaport-3 digital recorder, TEMEC Instruments B.V., Kerkrade, The Netherlands). Twelve EEGs, two electrooculograms (EOG), one submental electromyogram (EMG) and one electrocardiogram (ECG) signal were recorded. All signals were on-line digitized (12 bit AD converter, 610 $\mu$V/bit; storage sampling rate at 128 Hz for the EEG) and digitally filtered at 30 Hz (4th order Bessel type anti-aliasing filters, total 24 dB/Oct.) using a time constant of 1.0 s. EEG artefacts were detected by an automated detection algorithm (CASA, 2000 Phy Vision B.V., Kerkrade, The Netherlands). The EEGs were referenced against linked mastoids off-line subjected to spectral analysis using a fast Fourier transform (FFT, 10% cosine 4-s window) resulting in a 0.25 Hz bin resolution (26). SWA during non-rapid eye movement (NREM) sleep in the frequency range from 0.75 to 4.5 Hz was
Thermophysiological correlates of sleepiness

averaged per 2 hourly intervals throughout the nights. In order to analyze the time course of sleep EEG power density, the 8 h sleep episodes were subdivided into 2 h intervals after lights off.

**Data Analysis**

The statistical packages StatView™ 5.0 and SuperANOVA™ (Abacus Concepts, Berkeley, California, USA), and STATISTICA 6™ for Windows (StatSoft Inc., Tulsa, USA) were used. Raw data from each subject were inspected visually, and data segments that were affected by removal or malfunctioning of the temperature sensors were removed. These missing data (<0.5%) were replaced by values derived from a linear interpolation procedure. To reduce short-term fluctuations and the number of time segments, data were averaged either in 5min bins [used for detailed analysis of time courses within a nap (75/150min sleep/wake) cycle] or in 15min bins (used for the overall analysis over 37.5h). Since the protocols were identical for the first 150min after baseline sleep, the analysis was performed only for the following 37.5 hours.

The circadian time course was analyzed as follows: original data were purified from sleep/wake cycle-induced changes by subtracting the effects of mean nap cycles after data folding in 10*225min cycles. Before averaging, naps at a similar circadian phase were first averaged (NAP# 1 and 7, 2 and 8, 3 and 9, 4 and 10). In order to adjust for individual levels, the mean of the last 30min data (the last two 15min bins) of the weighted mean nap cycle was taken as zero (at these time point sleep inertia from the preceding nap had disappeared). The circadian time course was analyzed using this purified data set by cross-correlation analysis (according to 12). In order to purify original sleepiness data from additional long-term trends (see Results) residuals to a linear regression line were taken for the cross-correlation analysis. Cross-correlations were calculated for time lags between ±480min. Time lags of maximum and minimum r-values were extracted from smoothed (225min moving average) individual curves. Mean cross-correlation curves were calculated after Fisher’s Z-transformation and re-transformed for Figure 3.

The association between the time course of subjective sleepiness ratings and temperature data with respect to sleep inertia was calculated using a multiple regression analysis for repeated measures. The
between-subjects differences were taken into account, as well as the NAP#, using dummy coded subjects and NAP# as forced variables in the model (20). Temperature values (5min-bins) were taken at the same time as KSS ratings and MEL samples. Correlations between DIST, PROX, CBT or MEL and sleepiness were calculated for the 6 time points of the 10 x 75/150min sleep/wake cycle (60 values/subject).

Analyses of time courses were performed by one-, two- and three-way analyses of variance for repeated measures (rANOVA). All \( p \) values derived from rANOVAs were based on Greenhouse-Geisser 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 (12) were calculated.

In a first step we analyzed the data with respect to differences between the protocols (PROT, SD vs. NP), the time course within a nap cycle (TIME, e.g.15 x 15min temperature-bins/nap) and the number of nap-cycles (NAP#, ten nap cycles). Based on the finding that in all variables no significant 3-way interaction term (TIME\( \times \)NAP#\( \times \)PROT) was found (see below) we analyzed in a second step the effects within a mean nap-cycle in more detail with a higher time resolution of the temperature measurements [2-way rANOVA; TIME (5min-bins) \( \times \) PROT]. Third, in order to compare the circadian phase between the variables and between the protocols we carried out cross-correlation analyses. In the last section we analyzed thermoregulatory effects during an 8 hours sleep episode under different sleep pressure conditions (high or low levels of SWA).

RESULTS

Comparison between the two protocols (SD vs. NP)

This section describes the results for each variable between 1.5 – 40 hours after the 8 hours baseline sleep episode (Figure 1). The raw data of all temperatures was averaged in 15min-bins over both the SD and NP protocol. The only difference between the two protocols was that sleep was allowed in the 75min lights off episodes during the NP (statistical analysis thereof in Table 1). All data within the first 150min time segment after the baseline night did not statistically differ with respect to SD and NP (statistics not shown), indicating similar starting conditions before the two different protocols
Thermophysiological correlates of sleepiness

began [i.e. the first nap cycle (NAP#1) started with a 75min scheduled nap in dark followed by 150min scheduled wakefulness in <8 lux, etc.].

Core body temperature (CBT)
CBT mean values over the 37.5 hours did not differ between SD and NP (main effect: PROT: n.s.). The main effect NAP# was highly significant with highest values in NAP #3 and #9 and lowest values in NAP #5-6 describing the circadian time course. The significant interaction term TIME x NAP# reflects CBT changes during the naps which were strongly influenced by the uprising or falling part of the circadian CBT rhythm. During nap cycles only minor non-significant differences in CBT occurred between NP and SD. In comparison to SD, NP showed the largest reduction in CBT in the middle of the light phase (for detailed analysis see below Figure 2), but did not reach statistical significance.

Proximal skin temperature (PROX)
PROX mean values over the 37.5 hours did not differ between SD and NP. The significant factor NAP# showed highest values in NAP#1,2,3 and #9, and lowest value in NAP#5. Significant changes occurred during nap cycles with highest SD vs. NP differences in the middle of the dark phase and smallest differences at the end of the light phase (TIME x PROT sign.; for detailed analysis see below Figure 2).

Distal skin temperature (DIST)
All three main effects (PROT, TIME and NAP#) reached levels of statistical significance. Compared with SD, the main effect PROT showed elevated DIST mean values over the 37.5 hours in NP. However, this elevation in DIST was modulated during the nap cycle. Highest SD vs. NP differences were found in the middle of the dark phase and smallest differences at the end of the light phase within each nap cycle (for detailed analysis see below Figure 2). The NAP# x PROT interaction showed only a trend to statistical significance (p<0.1), indicating only small changes between NP and SD with respect to preceding duration of the protocols. The significant interaction term TIME x NAP# reflects changes during the naps which are strongly influenced by the uprising or falling part of the circadian rhythm in DIST. The time course of DPG (data not shown) was very similar to DIST (statistics in Table 1).
Salivary melatonin (MEL)

MEL mean values over the 37.5 hours did not differ between SD and NP. The main effect NAP# was highly significant with highest values in NAP #4-7 and lowest value in NAP #1-3 and NAP #8-10 describing the circadian time course. The significant interaction term TIME x NAP# reflects MEL changes during the naps which are strongly influenced by the uprising or falling part of the circadian MEL rhythm. During the scheduled wakefulness episode within the nap cycles only minor non-significant differences occurred between NP and SD, indicating negligible effects of light (<8 lux) on melatonin secretion after a 75min dark episode.

Subjective sleepiness (KSS)

Subjective sleepiness showed clear differences between the two protocols with respect to NAP# and TIME. All three main effects were statistically significant, however, significant two-way interactions indicate more complex interpretations. The sleep deprivation led to an increased mean sleepiness level of 1.2 units compared to NP, however, this difference was clearly modulated by the circadian rhythm (for detailed analysis see below Figure 2). The significant interaction NAP# x PROT reflects the gradual increase of sleepiness in SD with proceeding duration of wake time as well as a circadian component, and a different, circadian pattern in NP with maximum values at NAP# 5 & 6.

Nap induced effects (Figure 2)

This section describes the results of the nap-induced effects in a mean 75/150min sleep/wake cycle in 5min bins (Figure 2). In order to adjust for inter-individual differences data of the last 30 min of the light cycle were taken as zero (see Methods). As there were no significant 3-way interactions in any of the variables (Table 1), detailed 2-way ANOVA’s (factors PROT and TIME) could be performed. To study in detail the nap induced effects, 45 x 5min-binned data (total 225min) were analyzed (each bin previously averaged over all 10 nap cycles, see methods). Nearly identical statistical results were obtained as shown in Table 1 with 15min bins, therefore, a detailed ANOVA table is not shown.

A significant TIME effect in CBT reflected the influence of the protocols (however, the maximal variations were only 0.05°C). The apparent difference (0.05°C) between NP and SD in the middle of the light phase of a nap cycle did not reach statistical significance. PROX showed a significant TIME
x PROT interaction. In NP, PROX was significantly increased 10 min after lights off (maximum Δ0.5°C), and 5 min after lights on already decreased (no significant differences between SD and NAP). DIST also showed a significant TIME x PROT interaction. In NP, DIST was significantly increased 10 min after lights off (maximum Δ1.7°C at the end of the dark phase). This elevation in DIST during the dark phase of a nap cycle thereafter steadily declined until the end of the light phase (with no differences between the two protocols after 25min).

Sleepiness showed highest SD vs. NP differences at the beginning of the light phase (sleep inertia) and lowest at the end [TIME x PROT, F(5,35)= 12.28, p<0.0001]. In NP, highest sleepiness ratings were found 5min and lowest values 120min after lights on. SD showed small but significant modulations of sleepiness during the 225min -cycle [TIME, F(7,49)= 11.64, p<0.001]. Since sleep was not allowed these variations can be related to the various tasks within the protocol.

A multiple regression analysis for repeated measures revealed that the intra-individual time course of subjective sleepiness was significantly correlated with DIST (std b=+0.293, p<0.0001) and CBT (std b=−0.132, p<0.005) but not with PROX (std b=+0.055, n.s.) and MEL (std b=−0.041, n.s.).

Comparison between circadian time courses

In order to compare the circadian time course of SD and NP it was necessary to purify the data from the significant nap cycle-evoked effects (see Data analysis and Table 1). Therefore data of the weighted mean nap cycle data were subtracted from the original data (see methods). The resulting residuals include changes induced by e.g. circadian modulation and sleep pressure but no longer by nap-evoked effects (e.g. the increased skin temperatures after lights off, sleep inertia effects). All temperature variables were successfully detrended from the nap cycle-evoked effects as indicated by non-significant statistical ANOVA-terms for PROT, TIME and TIME x PROT (F and p values in Table 1 of NAP#, TIME x NAP#, NAP# x PROT and TIME x NAP# x PROT did not change, data not shown). Residuals of DIST, PROX and CBT followed a significant circadian pattern, CBT and PROX with inverse phase relationships to DIST (see below), all being similar for both protocols. Although sleepiness was significantly detrended (as indicated by non-significant statistical ANOVA-
Thermophysiological correlates of sleepiness

terms for TIME, PROT x TIME), the protocols remained different [PROT: F(1,7)=36.6, p<0.0005] and NAP# x PROT [F(9,63)=15.7, p<0.0001]. In NP, sleepiness clearly follows a circadian pattern with maximal values at NAP# 5 and 6. In SD, the time course of sleepiness shows a circadian pattern, which is overlapped with an increasing trend on the second day (for detrending of this effect see below).

In order to compare circadian phase relationships between the variables or protocols cross-correlation analyses were performed (see Table 2). Because subjective ratings of sleepiness showed a significant increase during SD (see above and Figure 1), KSS data were detrended by linear regression analysis (see methods). For no variables was a significant phase lag between NP and SD curves observed, indicating no differences in circadian phase between the two protocols. The phase relationships between DIST, PROX, MEL and KSS in comparison to CBT are shown in Figure 3. In NP and SD, no phase differences between KSS and CBT curves were found, indicating inverse phase locked patterns. Similarly, no phase differences were found between PROX and CBT. In contrast, DIST and MEL compared with CBT showed similar significant phase advances by 113 and 130min, respectively. Similarly, DIST and MEL were also phase advanced with respect to KSS [+96±29min, F(1,7)=10.7, p<0.05 and +114±40min, F(1,7)=8.27, p<0.05].

Comparison between baseline-, “second”- and recovery- night (Figure 4)

In order to disclose differences between the two protocols in the time course of temperatures during nocturnal sleep in the first (baseline, BN) and third (recovery RN) night, as well as during the “second” night (“night 2” = without sleep in SD, 2 short sleep episodes in NP; nap-purified data were taken), 30min-binned data of CBT and skin temperatures were analyzed by 3-way rANOVA (Table 3). This analysis tests the effects of low vs. high sleep pressure (NP vs. SD) on thermoregulatory changes within an 8h nocturnal recovery sleep episode in relation to slow wave activity measured in NREM - sleep. Additionally, the comparison with “night 2” allows an analysis of thermoregulatory effects with or without sleep at the same circadian phase (to simplify matters an endogenous circadian phase of 24 hours was assumed (13).
There were no significant differences between the two protocols in CBT, DIST and PROX (Table 3). Significant main factors of TIME and NIGHT were found. The two protocols did not differ between BN and RN allowing a combined testing against “night 2” (2-way rANOVA: BN vs. RN and TIME, n.s.). In “night 2” overall higher values in CBT (+0.18°C ± 0.04, p<0.005; maximal difference 5h after lights off: +0.31°C ± 0.05; difference at the end of the 8h sleep episode: +0.18°C ± 0.05) and lower values in DIST and PROX (DIST -0.96°C ± 0.11, p<0.05; PROX -0.31°C ± 0.10, p<0.05) occurred. The first significant reduction of CBT was found 2 hours after lights off (-0.08°C ± 0.03) and thereafter.

The time course of DIST and PROX during BN and RN did not statistically differ from “night 2”, indicating a general up-regulation of DIST and PROX after switching lights off (see above), even though DIST and PROX tended to increase faster during the first 2 hours when subjects were asleep [BN, RN vs. “night 2” x TIME, F(16,112)=2.70, (p<0.1)].

The time course of SWA during BN did not differ between the two protocols. Both curves declined in an exponential manner. In comparison to NP, SD showed a clear increase in SWA at the beginning of RN, confirming the well-known rebound effects of sleep deprivation on SWA. This increase gradually disappeared until the end of RN (fourth 2h- time segment: SD vs. NP, n.s.).

Taken together, during the BN (including the succeeding 150min light phase, statistics not shown), all measured variables did not differ with respect to the two protocols, indicating similar starting points. During the recovery night, SWA activity was increased in SD after 40h sleep deprivation, however, CBT, DIST and PROX were not changed. In comparison to the time segment at the same circadian phase without sleep (“night 2”), an 8 h- sleep episode increases DIST and PROX and conversely reduces CBT.

**DISCUSSION**

This nap vs. sleep deprivation protocol under constant routine conditions provided the necessary controlled situation for measuring changes in thermoregulatory variables related to time of day, sleep pressure, sleep itself, and after waking from a nap. Reducing masking effects of behavior and light
allowed a partial separation of the influence of the three processes determining sleepiness (2, 18) and their relationship to the thermoregulatory system. We have found an important difference between the circadian and homeostatic processes in this respect. This study supports our previous hypothesis that the circadian modulation of sleepiness is primarily related to the circadian regulation of distal vasodilatation (and hence to heat loss and CBT reduction), whereas the homeostatic regulated increase of sleepiness is nearly independent thereof (32). The findings further suggest that this close relationship between distal vasodilatation and sleepiness also holds for the process of sleep inertia, thus confirming and extending our published data (30). This means that both the evening increase in sleepiness, which leads to maximum sleep propensity in the middle of the night, and the exponential decline of sleepiness upon awakening, can be described as a function of changes in distal vasodilatation. In contrast, the homeostatic increase in sleepiness related to duration of prior time awake is not related to a thermoregulatory function. This homeostatic build-up process of sleepiness has been related to topographic EEG correlates in the frontal cortex (10). Based on these different physiological correlates, two "kinds" of sleepiness can be postulated (thermoregulatory-related and -unrelated).

The following discussion is structured with respect to the three-process model of sleepiness as related to thermoregulation. Our study permits comparison of the build-up of sleepiness (or sleep propensity) and its disappearance during sleep. We have presented DIST and PROX data to show the thermoregulatory effects as it occurs, and not the derivate DPG, which has been shown to be a reliable predictor of sleep onset propensity.

**Thermoregulatory effects related to circadian and homeostatic regulation of sleepiness**

The 40h sleep deprivation CR protocol has the disadvantage that the endogenous circadian modulation cannot be separated from the influence of the homeostatic rise in sleep pressure. Any overt circadian rhythm measured during a CR, even with no obvious homeostatic rise or fall may still be a masked rhythm. Such a masking component could be either sleepiness, which could lead to distal vasodilatation, or the counter-actions taken by a subject to keep awake as sleep pressure increases which could lead to distal vasoconstriction. A non-association of the homeostatic rise in sleep pressure
during sleep deprivation with thermoregulatory changes (e.g. distal skin temperature) could be due to equilibrium of the two counter-regulatory mechanisms. The nap protocol (NP) was designed to reduce the build-up of sleepiness to a low level. Comparison of the two protocols therefore provides a good estimate of homeostatic sleep pressure. Sleepiness increased with increasing duration of time awake and reached significantly higher levels than in NP after 20 hours. Previous CR studies found significantly higher subjective sleepiness ratings after ca. 16 hours of wakefulness. The discrepancy can be explained by the fact that after 16 hours of wakefulness the circadian increase of sleepiness overlaps with the homeostatic increase of sleepiness. Subjects may not be able to adequately differentiate (add) these two “kinds” of sleepiness, which could lead to a non-additive interaction at this circadian phase.

The finding that the circadian pattern of all measured body temperatures did not differ between the two protocols indicates they were independent of the homeostatic build up of sleepiness (sleep pressure). This allows the conclusion that all the measured circadian patterns are not influenced by a masking process via the sleep homeostat, i.e. counteractions taken by a subject to keep awake. The build up process of sleep pressure over 40 hours has in fact no, or only minor thermoregulatory consequences. This finding confirms a previous CR study where we found non-significant changes in body temperatures (CBT, PROX and DIST) at the same circadian phase 24h after a sleep deprivation episode (36). This does not preclude the possibility that with longer than 40h SD -protocols the thermoregulatory system may no longer be independent of sleep pressure.

PROX exhibited a circadian profile similar to CBT, whereas DIST showed an inverse circadian pattern phase advanced by 113min. As previously noted (36 and Fig.1), the falling limb of the CBT rhythm in the evening is steeper than the rising limb in the morning. Similarly, the rising limb of the DIST and DPG (data not shown) rhythm in the evening is also steeper (Fig.1) than the falling limb in the morning. This indicates an asymmetrical regulation of heat loss and heat production in the evening and morning. Heat loss seems to be dominant in the evening, heat production in the morning (36). We could also define the temporal relationship between the circadian rhythm of sleepiness and the thermoregulatory system. Sleepiness was significantly phase delayed by -96min and -114min with respect to DIST and MEL, and phase locked to CBT with a negative correlation coefficient. Because the circadian rhythm of DIST precedes both sleepiness and CBT, this could be the reason why DIST is...
a better predictor for sleepiness (and hence for sleep onset latency), than CBT (30, 31). Interestingly, sleepiness shows not only a close phase relationship to DIST and CBT but also a similar asymmetrical circadian pattern, with a faster rise in the evening than decline in the morning.

The phase relationship between the circadian rhythm of DIST and CBT may also provide a thermophysiological explanation of the so-called “wake maintenance zone” in the evening just before endogenous melatonin secretion and distal vasodilatation begins (39). At this circadian phase the circadian system counter-regulates with high effort the homeostatically increased sleepiness and sleep pressure to maintain wakefulness (17). In thermophysiological terms, the “wake maintenance zone” can be characterized as the most vasoconstricted state of distal skin regions in relation to CBT over the entire circadian cycle (i.e. low inner heat conductance with high CBT and low DIST, 4).

**Thermoregulatory effects related to lights off in a nap**

As found in earlier studies (5, 29, 30, 34, 37) thermoregulatory changes induced by lights off do influence CBT, but slowly. In contrast, distal and proximal skin temperatures increase immediately after lights off and before the onset of sleep stage 2 (5, 29, 30, 34, 37). This indicates that redistribution of body heat from the core to the shell occurs shortly after lights off via relaxation.

The well-known circadian modulation of sleep onset latency (SOL to sleep stage 1 or 2; 16) yielded shortest values near the CBT trough in NAP#5-6 and longest values in NAP#4 and 10 (42) just before distal skin temperature increased and CBT decreased. Similarly, an analysis of the time awake during a nap revealed maximal time awake values in NAP# 4 and 10 and minimal values during NAP#5-6 (42). However, independent of the differences in SOL and time awake in each nap, the skin temperatures increased at a similar rate (see Fig. 1), indicating that the subjects relaxed after lights off, however without necessarily falling asleep. An important factor for the relaxation-induced effects could be eye closure occurring before sleep onset, which deserves to be investigated separately.

The extent of the increase in DIST exhibited a trend towards circadian modulation; the non-significance can be explained by a ceiling effect. When DIST has already attained its circadian maximum - it is difficult to increase DIST above PROX. The large and rapid increase in skin
temperatures after lights off (0.7°C in PROX and 1.6°C in DIST) only led to small decreases in CBT (ca. 0.027°C / 75min) with a time lag such that the lowest value actually occurred 75min after lights on (Figure 2). Therefore, it is not possible to extrapolate the reduction of CBT from a short sleep episode (75min) to that during an 8 hour dark (sleep) episode (see below). Furthermore, had one measured subjective ratings of sleepiness after lights off until occurrence of sleep, one could speculate that it would increase in parallel with relaxation, withdrawal of the sympathetic nervous system, redistribution of heat from the core to the shell and increase of DIST. Thus, these changes after lights off can be interpreted as an inverse process of sleep inertia (see below). What we see are thermoregulatory effects mainly induced by relaxation, most probably via a decrease in sympathetic tone, and not by the occurrence of sleep per se (33). In real life situations it can be assumed that many effects on sleepiness and sleep, induced, e.g. by exercise, hot or warm bathing, intake of food, hot or cold fluids etc., may occur via redistribution of heat from the core to the shell or vice versa.

**Thermoregulatory effects related to sleep inertia**

After lights on, we found converse thermoregulatory effects to those after lights off (see above). Because no significant suppression of melatonin secretion was found after lights on (<8 lux), these effects cannot be explained by alterations in the retino-hypothalamic-pineal axis. Redistribution of blood from the shell to the core occurs via vasoconstriction, mainly in distal skin regions. However, this takes a while - in the hands faster than in the feet (data not shown). It is most rapid in proximal skin regions. Sleep inertia, as measured by sleepiness (22, 24, 34), shows a close temporal relationship exclusively with DIST, but not with CBT and PROX. A significant intra-individual correlation of the time course between DIST and sleepiness reveals a close functional relationship between the decay of sleep inertia and distal vasoconstriction. Most interestingly, we did not find a circadian modulation either in DIST or in sleep inertia(as measured by subjective sleepiness) after naps taken throughout the 24 hours in spite of large difference in sleep duration within a nap independent on circadian phase (42). This finding supports the notion that sleep per se is not crucial for sleep inertia (22, 34). We could show in two sets of experiments with either a nocturnal sleep episode (between 11 p.m. and 07 a.m.) or an afternoon nap (between 4 and 6 p.m.) that vasodilatation of hands and feet increased after lights off and that this was reversed after lights on. The time course of the distal skin temperature was
significantly and positively correlated with subjective sleepiness, reflecting similar temporal relationships in both studies. The extremities cooled at a rate very closely parallel to the decay of sleepiness. The symmetry between the thermoregulatory processes related to the increase in sleepiness and those related to its dissipation is striking. However, in order to directly test these relationships, further studies with thermophysiological interventions (e.g. cooling the extremities) are required. Based on these findings, it can be hypothesized that redistribution of heat from core to shell during sleep could actually counteract the waking signal that grows as sleep pressure declines (mirrored in the SWA decline). This inertia may help to maintain sleep or to fall asleep again more easily after a wake bout.

Effect of an 8h sleep episode on thermoregulation

If we compare the same circadian phase awake and asleep, the maximum reduction of 0.31°C occurs after 5 hours of sleep. In a similar CR-study a reduction of 0.46°C was found (5). The discrepancy can be explained by the higher light intensity level during the wake situation (40 lux vs. <8 lux in our study) leading to a suppression of melatonin secretion and consequently to higher CBT values. The CBT minimum during sleep was masked by the process of redistribution of heat from the core to the shell, initiated immediately after lights off and not by sleep per se (see above). In the first 2.5 hours after lights off, DIST and PROX increased to a peak with a similar maximum value. Sindrup et al. (1991) described this hyperaemic reaction as an effect which occurs with sleep. However, our method has a much higher time resolution than the radio-isotope method used by these authors, and thus we could clearly separate the relaxation from the sleep-induced effects. This leads to the conclusion that onset of sleep stage two, and herewith onset of increased slow wave activity, does not have additional thermoregulatory effects (see also 35). We have also shown that after lights off, when distal vasodilatation increases, heart rate falls in parallel, indicating a decrease in cardiac output, which could explain why the increase in distal vasodilatation does not induce an efficient heat loss, with a consequent decrease in CBT (35). Furthermore, after the acute hyperaemic response to lights off, PROX and DIST remained at a comparable high value, indicating a loss of the core-shell dichotomy (4) of the body during sleep. This one-compartment-state of course makes the body more vulnerable to heat loss or heat uptake, which could be a reason that a thermoneutral environment is preferred for sleep as a protection against external cooling and warming.
Effect of high and low sleep pressure on thermoregulation in the succeeding 8h sleep episode

In addition to the nap-related evidence, the nocturnal recovery sleep provided conclusive evidence that increased slow wave activity (particularly during the first 4 hours) that resulted from increased sleep pressure does not influence the thermoregulatory system. Previous studies claimed that high SWA has a thermoregulatory role, e.g. down regulation of CBT for energy conservation (40, 43, 44). Dijk et al. (1993) found the inverse, a relative increase of CBT during the recovery sleep after a 40h CR. These findings unfortunately cannot be used as arguments since none of them used posture-controlled protocols. Our protocols allow not only comparisons between BL and RN of a complete 40h CR under controlled body position, but also a comparison of the RN following conditions of relative high vs. low sleep pressure (SD vs. NP). This latter comparison controls for any possible confounding effect of the long period of 54h lying in bed on the influence of SWA on thermoregulation.

Taken together, this is the first study that details the complex interactions between circadian phase, sleep pressure and thermoregulatory effects in healthy young subjects kept under controlled posture, feeding and environmental conditions. We have disproved the long-held belief that sleep, or more precisely non-REM sleep, causes CBT to decline. Since ‘lying down’ and ‘relaxation after lights off’ evoke an increase in skin temperatures and a decline in CBT, these major masking effects have confounded prior studies and render their conclusions doubtful. This is not to deny the importance of such masking in real–life conditions. Thus, it appears that the circadian pacemaker drives the circadian propensity for sleep via a circadian rhythm in heat loss (vasodilatation). A similar, but inverse mechanism is responsible for the sleep inertia upon awakening. These thermoregulatory mechanisms underlying circadian sleepiness and sleep inertia are not related to changes in ‘homeostatic sleepiness’ resulting from being awake over longer periods.

PERSPECTIVES
The corollary of these findings has important consequences in sleep medicine. Independent of circadian phase, sleepiness is augmented by conditions of warming, and diminished by cooling of distal skin regions (leading to increased and reduced convective body heat loss, respectively). This knowledge supports the simple old-fashioned methods to speed up falling asleep (e.g. warm bath) or to rapidly dissipate sleep inertia (e.g. cold shower). In contrast, sleepiness related to extended episodes of prior wakefulness cannot be thermally manipulated, requiring a different strategy such as a short nap to diminish sleep pressure (a “power nap” that does not induce subsequent sleep inertia).

REFERENCES


   Sequence learning depends on sleep, the level of sleep pressure and circadian phase. *J Sleep Res* 11, Supp 1: 30-31, 2002.


Thermophysiological correlates of sleepiness


Thermophysiological correlates of sleepiness


**ACKNOWLEDGEMENT**

The study was supported by the Swiss National Science Foundation (SNF# 3130-054991.98/3100-055385.98 to C.C.). The authors thank Claudia Renz, Marie-France Dattler and Giovanni Balestrieri for their help in data acquisition, Drs. Alexander Rösler and Tobias Müller for medical screenings, Dr. Eus van Someren for his valuable comments and the subjects for participating.
Thermophysiological correlates of sleepiness

TABLES

Table 1:
Statistical analysis of thermoregulatory variables and sleep pressure across the two protocols.
3-way rANOVAs with factors: protocol (PROT, SD vs. NP), time within a nap cycle (TIME, 15 x 15min-bins/nap) and nap number (NAP#, ten nap cycles). MEL and KSS were measured only during wake the phase, therefore, df of factor TIME is 5 for these variables.

<table>
<thead>
<tr>
<th>FACTOR</th>
<th>CBT</th>
<th>PROX</th>
<th>DIST</th>
<th>MEL</th>
<th>KSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROT</td>
<td>F(1,7)</td>
<td>0.59 n.s.</td>
<td>1.54 n.s.</td>
<td>23.7 p&lt;0.005</td>
<td>F(1,7)</td>
</tr>
<tr>
<td>TIME</td>
<td>F(14,98)</td>
<td>4.41 p&lt;0.05</td>
<td>19.9 p&lt;0.0001</td>
<td>18.2 p&lt;0.005</td>
<td>F(6,35)</td>
</tr>
<tr>
<td>NAP#</td>
<td>F(9,63)</td>
<td>3.12 p&lt;0.0001</td>
<td>3.44 p&lt;0.05</td>
<td>11.3 p&lt;0.0001</td>
<td>F(8,63)</td>
</tr>
<tr>
<td>TIMExNAP#</td>
<td>F(126,882)</td>
<td>12.7 p&lt;0.0001</td>
<td>1.17 n.s.</td>
<td>5.43 p&lt;0.005</td>
<td>F(45,315)</td>
</tr>
<tr>
<td>TIMExPROT</td>
<td>F(14,98)</td>
<td>2.40 n.s.</td>
<td>11.9 p&lt;0.005</td>
<td>25.9 p&lt;0.0001</td>
<td>F(6,35)</td>
</tr>
<tr>
<td>NAP#xPROT</td>
<td>F(9,63)</td>
<td>0.51 n.s.</td>
<td>0.50 n.s.</td>
<td>3.14 (p&lt;0.1)</td>
<td>F(8,63)</td>
</tr>
<tr>
<td>TIMExNAP#xPROT</td>
<td>F(126,882)</td>
<td>1.10 n.s.</td>
<td>1.01 n.s.</td>
<td>1.20 n.s.</td>
<td>F(45,315)</td>
</tr>
</tbody>
</table>

Table 2:
Phase relationships between variables and between protocols.
Maximum or minimum lags were extracted from individual cross-correlation curves (mean ± sem, for explanation of abbreviations see Figure legend 3; + lag-values indicate phase advances and - lag-values phase delays).

<table>
<thead>
<tr>
<th>Variable</th>
<th>SD vs. NP</th>
<th>t-test vs. CBT</th>
<th>vs. CBT</th>
<th>vs. CBT</th>
<th>t-test (vs. 0-lag)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBT</td>
<td>-0.09±0.38</td>
<td>n.s.</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>DIST</td>
<td>+0.47±0.56</td>
<td>n.s.</td>
<td>+1.89±0.84</td>
<td>+1.87±0.56</td>
<td>1.88±0.46</td>
</tr>
<tr>
<td>PROX</td>
<td>+0.85±0.47</td>
<td>n.s.</td>
<td>-0.26±1.58</td>
<td>-1.12±1.72</td>
<td>-0.69±1.25</td>
</tr>
<tr>
<td>KSS</td>
<td>-0.35±0.71</td>
<td>n.s.</td>
<td>+0.17±0.38</td>
<td>+0.38±0.51</td>
<td>+0.27±0.29</td>
</tr>
<tr>
<td>MEL</td>
<td>+0.06±0.35</td>
<td>n.s.</td>
<td>+2.09±0.50</td>
<td>+2.24±0.57</td>
<td>+2.17±0.51</td>
</tr>
</tbody>
</table>
Table 3:
Statistical analysis of thermoregulatory variables and slow wave activity (SWA) across the two protocols by 3-way rANOVA.

<table>
<thead>
<tr>
<th>FACTOR</th>
<th>CBT</th>
<th>PROX</th>
<th>DIST</th>
<th>SWA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROT</td>
<td>F(1,7) 0.25 n.s.</td>
<td>1.31 n.s.</td>
<td>5.03 (p&lt;0.1)</td>
<td>F(1,7) 27.6  p&lt;0.005</td>
</tr>
<tr>
<td>TIME</td>
<td>F(16,112) 42.4 p&lt;0.0001</td>
<td>11.5 p&lt;0.0001</td>
<td>9.21 p&lt;0.0001</td>
<td>F(3,21) 79.1 p&lt;0.0001</td>
</tr>
<tr>
<td>NIGHT</td>
<td>F(2,14) 8.19 p&lt;0.005</td>
<td>8.63 p&lt;0.05</td>
<td>34.7 p&lt;0.0001</td>
<td>F(1,7) 95.4  p&lt;0.0001</td>
</tr>
<tr>
<td>TIMExNIGHT</td>
<td>F(32,224) 5.97 p&lt;0.01</td>
<td>2.16 (p&lt;0.1)</td>
<td>2.44 (p&lt;0.1)</td>
<td>F(3,21) 1.02 n.s.</td>
</tr>
<tr>
<td>TIMExPROT</td>
<td>F(16,112) 0.80 n.s.</td>
<td>0.41 n.s.</td>
<td>0.57 n.s.</td>
<td>F(3,21) 5.88 p&lt;0.01</td>
</tr>
<tr>
<td>NIGHTxPROT</td>
<td>F(2,14) 1.87 n.s.</td>
<td>1.50 n.s.</td>
<td>0.21 n.s.</td>
<td>F(1,7) 44.1  p&lt;0.0001</td>
</tr>
<tr>
<td>TIMExNIGHTxPROT</td>
<td>F(32,224) 0.74 n.s.</td>
<td>1.22 n.s.</td>
<td>1.37 n.s.</td>
<td>F(3,21) 7.31 p&lt;0.001</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS:

Figure 1:
Mean time course of all measured variables of N=8 subjects for the entire protocol. Temperature data are binned in 15-min intervals. For better visualization s.e.m. values were omitted (mean s.e.m.-values/time bin did not differ between SD and NP, data not shown). The large black and grey areas indicate dark phase (nocturnal sleep) for both protocols; the black and grey areas between 0 and 40h indicate dark phase only for NP.

Figure 2:
Time course (Δ mean ±s.e.m.; N=8 subjects; 5min bins) of all measured variables during an overall averaged 75/150min sleep/wake cycle. In order to adjust for inter-individual differences data of the last 30 min of the light cycle were taken as zero (see Methods). Asterisks indicate significant differences (p at least <0.05, see text) between the two protocols. Black and grey areas indicate dark phase.

Figure 3:
Cross-correlation curves (5min bins) between skin temperatures (proximal, PROX; distal, DIST), subjective sleepiness (KSS) and core body temperature (CBT). In order to receive equidistance in time for all variables, KSS and MEL were linearly interpolated. Mean (thick lines) ± sem (thin lines), after retransformation of Fisher’s z-values; N=8 subjects; black lines: SD, grey lines: NP. Note: DIST and MEL are significantly phase advanced by about 2h with respect to CBT (see Table 2).

Figure 4:
Time course (mean ±s.e.m.; N=8 subjects; 30min bins) of slow wave activity (SWA), distal- (DIST) and proximal- (PROX) skin temperatures, and core body temperature (CBT) during the baseline night (BN), the time segment on day two at the same circadian phase (“night 2”) without sleep and the recovery night (RN). Note: In contrast to SWA, no significant differences were found in any body temperature measures between NP and SD. Asterisks indicate significant differences between the two protocols (p at least <0.05, see text). Black and grey areas indicate dark phase of nocturnal sleep.
Thermophysiological correlates of sleepiness

Figure 1

[Graph showing correlations between various physiological measures and sleepiness over time, with key indicators such as core body temperature, proximal and distal skin temperature, salivary melatonin levels, and sleepiness ratings.]
Figure 2

Thermophysiological correlates of sleepiness

- Sleepiness (Δ KSS units)
- Distal skin temperature (Δ°C)
- Core body temperature (Δ°C)
- Proximal skin temperature (Δ°C)
- Salivary melatonin (Δpg/ml)

Legend:
- SD
- NP

Note: * indicates significant difference (p < 0.05), n.s. indicates non-significant difference.
Figure 3

- **DIST vs. CBT**
- **PROX vs. CBT**
- **MEL vs. CBT**
- **KSS vs. CBT**

- advance delay

$r$-value

lag (h)

-8 +8 +4 0 -4 -8
Thermophysiological correlates of sleepiness

Figure 4