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Cardiac autonomic control, influenced by thermoregulatory changes in humans

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auf Antrag von Prof. Dr. Christian Cajochen
Prof. Dr. Joachim Hermsdörfer
Prof. Dr. Heinrich Reichert

Basel, den 21.06.2011

Prof. Dr. Martin Spiess
Dekan der Philosophisch-Naturwissenschaftlichen Fakultät

"Panta rhei"

Heraklit von Epheso

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Table of Contents

Summary

It is known that autonomic cardiac control and thermoregulation are connected. Heart rate variability (HRV) is an analysis which refers to the regulation of the sinoatrial node of the heart by the sympathetic and parasympathetic branches of the autonomic nervous system (ANS). Publications that investigate HRV under changed thermoregulatory states lack certain information e.g. time of day or measures for both, skin and core body temperature (CBT). Thus, the main focus of the present thesis is to broaden the basic physiological and chronobiological knowledge about the interaction between autonomic cardiac control and thermoregulation. To examine this subjects' thermoregulatory state was modified either by passive body heating (chapter 2) or was investigated in subjects with endogenous dysregulation of distal vessels, leading to thermal discomfort from cold extremities (chapter 3 and 4).

In chapter 2 both physiological and chronobiological aspects of a thermal load in the evening on CBT, melatonin, heart rate (HR) and HRV measures were addressed in healthy young men within a stringent controlled 40-hour 'constant routine' protocol. Passive body heating for 30 minutes was able to induce a phase shift of output rhythms of the suprachiasmatic nuclei (SCN), the origin of the endogenous circadian rhythm. HR, CBT and melatonin were phase advanced by ~2h, whereas no shift was analysed for HRV measures. These findings indicate either no influence of the SCN to measures for HRV or only peripheral clocks were shifted by the evening heat load without a contribution from the SCN. Further, the acute implication of heat to the cardiovascular system was of great interest. Not only was an increase in CBT, HR and a global vasodilated body state found, but also an increase of sympathetic and decrease of the vagal activity, and an overall reduced heart rate variability.

Most publications about HRV derive from studies in male subjects or from mixed gender groups. However, gender influences have been reported. The studies described in chapter 3&4 are conducted only in female subjects; this extends the previous knowledge of the interaction of cardiac and thermoregulatory control specifically for this group.

In chapter 3, HR and HRV analyses were assessed in a group of women including a different physiological thermoregulatory state and compared with the results derived from a control group of women during a stringent controlled 40-hour 'constant routine' protocol. Spectral analysis of RR-intervals during 3-minutes paced breathing episodes every 2-hour revealed lower activity in the high-frequency band but not in the low-frequency band. This is leading to an elevated sympathovagal balance in women with an endogenous

dysregulation of distal vessels. These findings indicate an autonomic imbalance in this study sample. From a chronobiological point of view a circadian rhythm for power in the low frequency band and for HR occurred. The power for the low frequency band was low in the afternoon and high at the end of the subjective night. Further, a correlation was found between sleepiness and sympathovagal balance in both groups. These findings suggest active regulation of the ANS against fatigue in women, regardless of their thermoregulatory state.

In chapter 4, HR and HRV were analysed in young healthy women during the sleep initiation period. This period is especially characterised by striking changes in HR, body temperature and electroencephalogram wave amplitude and frequency. Skin temperature, especially the gradient between distal and proximal skin temperature, is known to be an easily measurable variable for distal vasodilatation, which is controlled by the ANS. The relationship between skin temperature and cardiac autonomic control during sleep initiation was investigated. Furthermore, changes in sleep, temperature or cardiac regulation after constant posture conditions without prolonged wakefulness were analysed. Thus, the pattern of core body and skin temperature, HR and its variability was elucidated during the sleep initiation period, examined on two subsequent nights in the chronobiological laboratory. To prolong the period from lights off to sleep onset and hence to a vasodilated body state, data derived from subjects that had thermal discomfort from cold extremities and difficulties initiating sleep was used. Sleep onset latency was longer in the first night compared to the second. Hence, the faster decline of arousals in the second night compared to the first allowed a faster build-up of sleep stage 2, slow wave sleep and delta power. Both, proximal and distal skin temperatures showed an increase after lights out. The distal-proximal skin temperature gradient, used as a measure for distal vasodilatation, started with a lower level after lights out in the first night, compared to the second. To summarise, different dynamics and differences between the two nights in skin temperature and sleep variables (but not in HR and HRV variables) were examined.

In conclusion the present thesis provides several important outcomes. Of particular note is, firstly, that a heat pulse induces an acute thermoregulatory state. This leads to physiological changes indicating that the heart is less adaptable to further alterations in environmental circumstances. Furthermore, a heat pulse in the evening induces a significant phase advance. No shift was seen in measures for HRV the day after the intervention. As such, were the SCN to be implicated in the CBT shift, it would exclude involvement of the ANS innervating the heart. Subjects that physiologically have a different

thermoregulatory state than controls, due to a dysregulation of acral vessels, manifest an autonomic imbalance. Hence, these subjects might be at a higher risk of developing heart failure. Further subjects with the same different physiological thermoregulatory state revealed that the central regulatory mechanisms of sleep or skin temperature and HR or HRV are rather weakly coupled. Last but not least, the present thesis adds new aspects to the ongoing debate about the VLF range of HRV. In this it seems very likely that VLF measures reflect not only thermoregulatory processes, but also vagal modulation.

The thesis provides evidence that the ANS modulating the heart (reflected by HRV measures) is hardly influenced by the SCN, but by thermoregulation.

Chapter 1

General introduction

Circadian rhythms

Almost all living organisms experience daily and/or seasonal changes of physiology and behaviour. From an evolutionary point of view, these variations enable organisms to temporally adapt to, and anticipate environmental alterations (e.g. the light-dark cycle of a day, or seasonal changes of a year, etc.). The daily reoccurrence of light and darkness represents the most systematic time cue on earth (summarised in Menaker 1969).

However, to investigate the precise mechanisms that underlie biological rhythms, and also to better understand how these rhythms influence physiological and behavioural responses, environmental time cues, such as the light-dark cycle, need to be controlled for. In a classical study conducted in 1938, Kleitman and Richardson investigated their own sleep -wake behaviour and body temperature, while living under a 28-h day in a cavern some hundred metres underground. The idea driving this experiment was to know if their rhythm was capable to adjust to a cycle out of the 24-h day (Kleitman 1963). Due to few data (N=2) and the lack of knowledge, that even low light intensities could act as a zeitgeber, the study provided mixed results. Nearly 20 years later, Aschoff and colleagues (Aschoff 1981) refined this experiment in an underground-bunker. Participants were asked to schedule their day according to their preferences. Aschoff elucidated a rhythm in humans, independent from external, environmental stimuli: an endogenous circadian (lat. about one day) cycle. He discovered that the free running -- unmasked period length tau (τ) of a cycle of e.g. core body temperature is close, but not exactly 24-h (25 +/- 0,56 h). Later studies with more stringently controlled conditions for light, time and social cues revealed a τ closer to 24-h (24.18 h; Czeisler et al. 1999; 24.07 +/- 0.33 h; Gronfier et al. 2007).

Due to the persistence of rhythms, even when external stimuli are minimized, an endogenous time keeping system (circadian pacemaker) has been suggested. Brain lesion studies in animals have shown that neurons in the suprachiasmatic nuclei (SCN) are involved in this timekeeping system (Stephan and Zucker 1972, Moore and Eichler 1972). More evidence for that reveal following findings: the SCN is where the entrainment to the “light-dark” pathway ends (for review: Moore et al. 2002); SCN lesions can disrupt the temporal pattern of the sleep-wake cycle and core body temperature (Cohen and Albers

1991); isolated SCN neurons preserve the circadian control of their neuronal firing rate (Reppert and Weaver 2002). Furthermore, transplanting fetal SCN tissue in a SCN lesioned animal can restore its rhythmic behaviour (Lehman et al. 1987). Taken together, a large body of evidence suggests that this small area, bilaterally located in the anterior hypothalamus, above the optic chiasm of the mammalian brain (Figure 1) is the origin of an endogenous circadian rhythm.

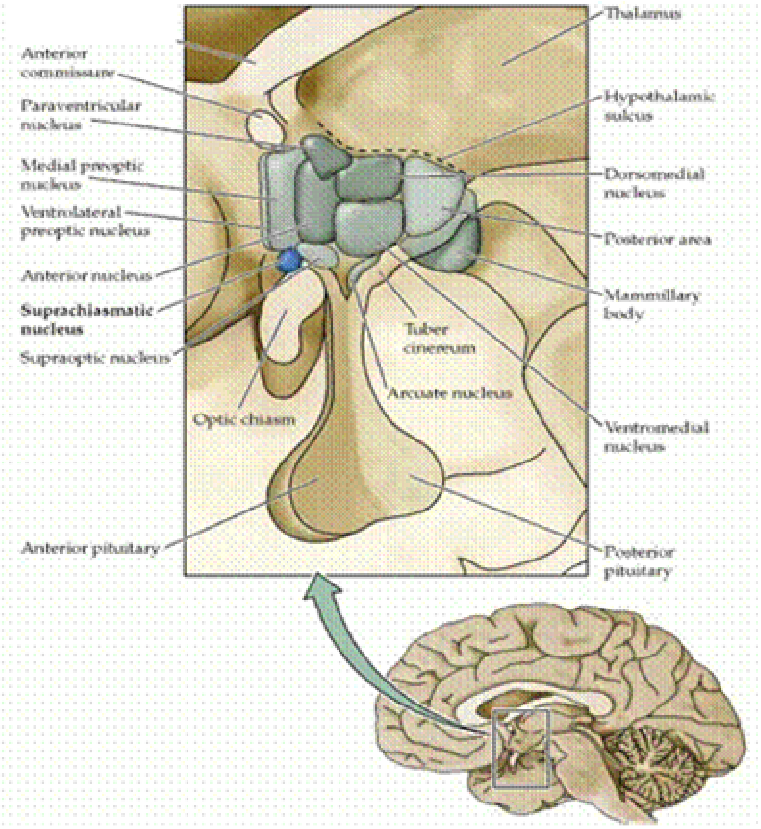


Fig. 1*: Localisation of the inner clock within the human suprachiasmatic nucleus (SCN).*
Modified from Purves 2001.

Explants of mammalian organs such as heart, skin, and retina etc. revealed the existence of periphery oscillators, including in extra-SCN areas of the brain (Tosini and Menaker 1996, Balsalobre et al. 2000, Yamazaki et al. 2000, Kaeffer and Pardini 2005, Abe et al. 2002, Ramanathan et al. 2010). Each peripheral oscillator is known to have tissue-specific differences in circadian phase and period (Yoo et al. 2004). However, the rhythm of the peripheral oscillators exhibits the 3 main properties of circadian clocks: it runs free in constant conditions, its phase is reset by zeitgeber pulses (isolated retina, Tosini and Menaker 1996) and it is temperature compensated (isolated tissue of mammalian pituitary gland, cornea, adrenal gland, and lung, Reyes et al. 2008). If embryonic fibroblasts that are deficient on a distinct clock gene and hence show short-period phenotype in vitro, are implanted into wild-type mice, the implanted clocks exhibit rhythmic gene expression with phase and period length close to those in the host peripheral tissues (Pando et al. 2002).

Thus, it is postulated that the whole system of the inner clock is an integrative system, with the master clock in the SCN, receiving input from the environment (e.g. light) and conveying timing information to peripheral clocks, located in various organs. Thereby the peripheral clocks are used to drive tissue-specific output programmes that are matched to local needs (Hastings et al. 2003).

Due to the fact that the endogenous period length τ varies from the 24 hours of a geophysical cycle of a day the rhythm is synchronised by so called 'zeitgeber', which have the ability to entrain the inner clock. Light, as photic zeitgeber, is the most potent signal for the human circadian system. Photons are received by specialized receptors (melanopsin) of the retina and transmitted via the retino-hypothalamic tract (RHT) to SCN neurons (Dai et al. 1998, Hannibal 2002, Ruby et al. 2002). In humans, light pulses given before the time of the core body temperature minimum elicit phase delays (i.e., activity starts later than expected the next day), while if given after the core body temperature minimum, it can trigger phase advances (i.e., activity starts earlier than expected) on the day following the light pulse (Minors et al. 1991). Although light is the most powerful synchronizer for the SCN, non-photic stimuli can influence and/or shift the SCN phase in rodents (Mistlberger and Skene 2005). For instance, when administered at supra-physiological doses during the subjective day, melatonin can induce phase advances of locomotor activity (Armstrong 1989). Moreover, a daily subcutaneous infusion of melatonin in dark conditions around the beginning of the subjective night can synchronize the SCN clock in nocturnal rodents (Pitrosky et al. 1999). Depending on time of day, phase shifts after an oral dose of melatonin or melatonin agonists have also been investigated in humans (Arendt and Skene 2005, Kräuchi et al. 1997a).

Further, food availability can induce changes in activity pattern in rodents, such that if food access is restricted to the light period, they will become active during the day in the hours preceding new food access (Damiola et al. 2000, Stokkan et al. 2001). In this regard exercise has been investigated as well (Atkinson et al. 2007). Because all those stimuli are integrated in different brain regions, it can be assumed that the SCN receives input from other brain areas (Moore 1996). Two other input pathways, than the RHT have been described, namely the geniculohypothalamic tract (GHT) and serotonergic input from the dorsal and median raphe nucleus (reviewed in Dibner et al. 2010).

To unravel the effects of the endogenous circadian rhythm on human physiology and behavior, "masking" effects (e.g. light, sleep, movement, etc.) need to be ruled out. These

effects obscure the information about phase and amplitude of the output of the SCN (e.g. bright light abolishes the circadian release of melatonin; or exercise and food intake elevates CBT). Thus, rhythms observed under ambulatory conditions are considered to be a mixture of endogenous- and masking components. Two important protocols have been developed in order to dissect out the relative contributions of circadian and homeostatic processes in humans, namely the constant routine and the forced desynchrony protocols (for review see Duffy and Dijk 2002). In the first procedure, subjects remain awake in a semi-recumbent posture for more than 24 hours (for at least one complete circadian cycle), which is referred as constant routine (CR) protocol with prolonged awakening. Thereby, the amplitude and phase of many circadian rhythms can be elucidated without the masking effects of motor activity, meals and lighting conditions. In the second protocol, subjects live on artificially very long or very short days, so that the circadian system is no longer entrained to the imposed sleep–wake cycle, enabling measurement of an individual's circadian period, known as CR with forced desynchrony (Mills et al. 1978, Czeisler 1990). The desynchronised subjects sleep at different circadian phases of the entire 24-h cycle, and subsequent analyses can differentiate the contribution of the sleep homeostat or the biological clock to a given variable (Dijk et al. 1997).
In summary, these stringent protocols provide powerful experimental tools to separate endogenous circadian patterns from various masking influences.

Output of the SCN and their relationships

Animal studies with injection of antero-and retrograde tracers revealed that targets for rhythmic information from the SCN are various other brain regions, and subsequently all peripheral clocks of the whole organism (Czeisler 1990, Silver et al. 1996, Vogelbaum and Menaker 1992, reviewed in Kalsbeek et al. 2006). Neural efferent pathways transmitting signals via multisynaptical fibres are known. Those neurons have been shown to terminate the paraventricular nucleus of the hypothalamus (PVN), the medial preoptic area, the preoptic anterior area of the hypothalamus (POAH) and the dorsomedial nucleus of the hypothalamus (DMH; Saper et al. 2005, Kalsbeek et al. 2006). These areas represent integrative nuclei from where fibres transmit to the ventrolateral preoptic nucleus (VLPO) or intermediolateral column of the upper thoracic cord (IML). These projections have been demonstrated to contribute to thermoregulation (POAH), melatonin secretion (PVN) or wake-sleep regulation (DMH, VLPO; Saper et al. 2005, Fig. 2).

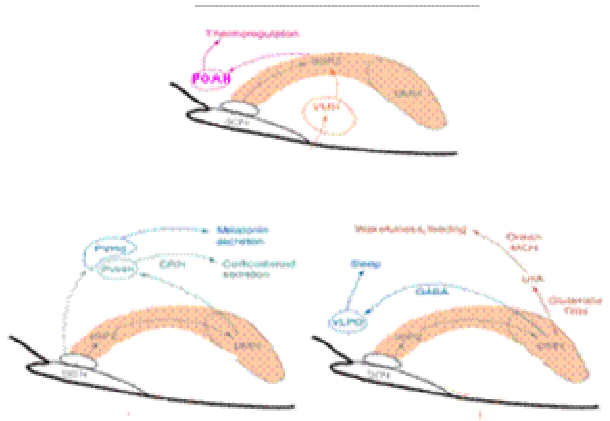


***Fig. 2**: Neural transmission pathway, starting from the suprachiasmatic nuclei (SCN); POAH = preoptic anterior area of the hypothalamus; PVN = paraventricular nucleus of the hypothalamus; DMH = dorsomedial nucleus of the hypothalamus; VLPO = ventrolateral preoptic nucleus.*
Modified from Saper et al. 2005.

Several neurochemicals have been suggested to be involved in the transmission of the rhythmic information, for example gamma-aminobutyric acid (GABA), glutamate, vasoactive intestinal peptide, vasopressin, etc. (reviewed in Dibner et al. 2010). To understand the precise effects of the neurotransmitters, it is necessary to consider their differential effects. Vasopressin, for example, acts inhibitory when derived from SCN neurons on the HPA axis activity but stimulatory when derived from PVN neurons (reviewed in Kalsbeek et al. 2010).
A further issue for research needs in this field is the observation that light-dependent synchronization is similar in both, nocturnal and diurnal animals. Thus, there is no difference in the phase of clock gene expression in the SCN, irrespective of diurnal or nocturnal behaviour (summarised in Dibner et al. 2010).

An example for a very promising marker rhythm evoked by the SCN is melatonin. The hormone is synthesized by pineal cells in the pineal gland out of tryptophan (Reiter 1991). Melatonin secretion occurs in a rhythmic manner with low levels during the day and high concentration during the night. GABA-ergic and glutamatergic output from the SCN are discussed to be responsible for the rhythmic fluctuation of melatonin secretion. More precisely, nocturnal low activity of inhibitory GABA-ergic input together with the continuously glutamatergic input are thought to activate the pre-autonomic PVN neurons that control the sympathetic input to the pineal gland to assure high melatonin concentration during the night. During light periods however, daytime activity of GABA-ergic SCN projections to the PVN have been reported to assure low melatonin concentration (Gupta 2007). Furthermore the SCN output to the pinealocytes controls the rhythm of the enzyme N-acetyltransferase, which is the limiting agent of the melatonin synthesis and therefore also contributes to the rhythmic secretion of melatonin (Bogumil 1974, Coon et al. 1995, Zeitzer et al. 2000).

When the administration of exogenous melatonin is well-defined timed, both a phase advance and a delay in the SCN-output occur (Lewy et al. 1992, Wirz-Justice et al. 2002, Rajaratnam et al. 2003). A number of melatonin (MT) receptors have been found in the SCN. It is suggested that the SCN MT1 receptors refer to the amplitude of the SCN circadian rhythm, while MT2 receptors are related to the entrainment of circadian rhythms (Liu et al. 1997, Hunt et al. 2001). This might indicate that *endogenous* melatonin stabilises the phase of the circadian rhythm by binding on the same receptors. Therefore and because masking effects have only minor influence on the melatonin rhythm (except light), endogenous melatonin is suggested to be the best marker rhythm for the timing of the internal clock in humans, at least in controlled studies (Klerman et al. 2002).

The melatonin concentration in serum or saliva starts to rise about 2 hours before habitual bed time (Lavie 1997) under entrained conditions. Thus, to emphasise the phase of the SCN mainly the dim light melatonin onset (DLMO; when the melatonin concentration reaches a special threshold level in the evening) is calculated. Other phase markers are e.g. the minimum of the core body temperature (CBT) or heart rate (HR) rhythm.

The specific temporal relationship of one oscillating system to another is called *phase of entrainment* (Roenneberg and Merrow 2007). Distinction of the phase of a cycle can be done by any reference point of the detected rhythm, like the CBT minimum, dim light melatonin onset (DLMO) or dawn/dusk. For example, the timing of the peak melatonin concentration is closely related to the nadirs of CBT or alertness (Rajaratnam and Arendt 2001), and sleep onset to an increase in distal skin temperature (Kräuchi et al. 1999a, Kräuchi et al. 1999b). One can discriminate between external and internal phase of entrainment (Roenneberg et al. 2003, Daan and Oklejewicz 2003, Kräuchi 2007b). One reference point within the external phase of entrainment origins from the zeitgeber, the other from the endogenous circadian rhythm, whereas the origins of both reference points within the internal phase of entrainment are from endogenous circadian rhythms. However, the result of internal and external phase of entrainment is a certain phase angle (Figure 3).

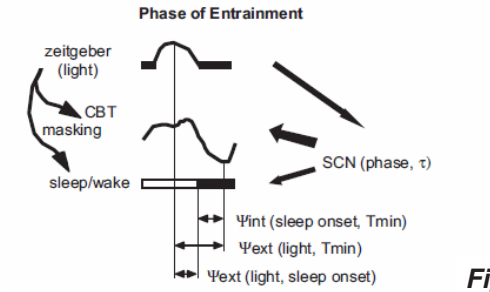


***Fig.3**: Phase of entrainment. Kräuchi 2007b.*

Phase response curves for different zeitgeber reflect the dependence on τ and/or the Zeitgeber rhythm T. The most developed relationship for phase and zeitgeber is existent for bright light exposure: At the end of the subjective night a phase advance, at the beginning of the subjective night a delay occurs. Light exposure during the subjective day has no effect on the phase of the human circadian rhythm. It seems likely, that a light signal has stronger phase shifts, the closer it occurs to the CBT minimum. The phase of entrainment is also dependent on the strength of the zeitgeber. A weak zeitgeber signal will lead to a small phase shift, whereas a strong signal will induce a big one, and a long zeitgeber signal will lead to a stronger phase shift than a short one. Furthermore the ability to get entrained is important (physiology of the receptors in the eye, functionality of the circadian pacemaker, etc.; Roenneberg et al. 2003, Kräuchi 2007b).

Even if the phase angle is not a fixed physiological parameter, it seems important not to deviate too much from an overall mean. Disturbances in the phase of entrainment might result in sleep problems (Vollenweider et al. 2008, Campbell and Broughton 1994, Murphy and Campbell 1997) or even cardiovascular diseases (Scheer et al. 2009).

A model of nature to elucidate chronically circadian misalignment is subjects suffering from vascular dysregulation (VD) combined with prolonged sleep onset. Vascular dysregulation characterises an inappropriate local regulation of the microcirculation, like arteries, veins, and capillaries. In contrast to the secondary VD, known as dysregulation due to a primary disease (like systemic lupus erythematosus or glaucoma), the primary VD (PVD) has another origin (summarized in Grieshaber et al. 2007). However, the leading symptom of the syndrome is suffering from cold extremities (hands and feet), known as thermal discomfort from cold extremities (TDCE). Additionally subjects react to stimuli such as cold, mechanical, or emotional stress with an intense vasoconstriction (Chai et al. 1999, Flammer 1992, Mahler et al. 1989, Yang et al. 2007). A further finding is that PVD is related to prolonged sleep onset latency (SOL; Pache et al. 2001). This finding was confirmed by a representative survey (Krauchi et al. 2008). A very controlled laboratory study, containing data about subjects complaining about TDCE and SOL in questionnaires, revealed not only polysomnographically estimated longer times falling asleep, but also a delay of the circadian system by approximately 1h compared to controls (Vollenweider et al. 2008).

The following sections will focus on the sleep wake cycle, body temperature, and heart rate, all of which are considered as marker rhythms of the SCN.

Sleep – Wake Cycle

The rest-activity cycle is used in many animal studies to investigate behaviour derived from the SCN, hence as marker rhythm of the main pacemaker (Stephan and Zucker 1972, Ralph et al. 1990). In humans the rest-activity cycle or sleep – wake cycle is confounded by overt masking effects, like drinking alcohol (leading to vasodilatation and hence to sleepiness), intake of caffeine (acts as adenosine-receptor-antagonist, thus, reduces sleep efficiency; Landolt et al. 1995) or acute effect of food intake (Lowden et al. 2004), just to mention a few. Nevertheless it served as a marker of the endogenous circadian clock function in isolation studies (Aschoff 1965, Aschoff 1994, Wever 1975), in phase-shift studies (Kronauer et al. 1982, Honma et al. 1995, Duffy et al. 1996) and in psychiatric studies (Wehr et al. 1983, Souetre et al. 1989, Teicher et al. 1993, Bromundt et al. 2011). Isolation studies revealed that the sleep – wake cycle in healthy young humans consists of ~7h sleep, if it can be chosen as preferred (Zulley et al. 1981). Ambulatory studies with subjects aged 20-59y have shown a subjective measured average sleep time of ~7.7h, depending on age and chronotype (Carrier et al. 1997). Thereby the chronotype characterises at what time of day individual physical functions, like the hormone level or body temperature reach a certain level or initiating sleep is preferred. In morning types the plasma melatonin rhythm, the temperature minimum and the clock time for initiating sleep occur earlier than in evening types (Duffy et al. 1999, Baehr et al. 2000, Lack L 2009). Interestingly, a correlation between chronotype and endogenous period length was shown in that early types have a shorter period length than evening types (Duffy et al. 2001). The habitual sleep wake cycle and hence the chronotype can not only be measured by subjective questionnaires (Morningness/Eveningness Questionnaire; Munich ChronoType Questionnaire), but objectively by actimetry, measuring acceleration, via a wrist worn watch-like device. Unfortunately the actogram provides no information about exact sleep cycles. Therefore the recording of electrical activity along the surface of the head produced by the firing of neurons within the brain is needed. Indeed, with this technology detailed information about sleep cycles could be revealed, but until now the exact function of sleep is still undiscovered. Sleep and sleep deprivation studies suggest that sleep has more than one function, like recovery at the cellular network and endocrine system levels, energy conservation, and a role in learning and synaptic plasticity (reviewed in Mignot 2008).

Human sleep is very heterogeneous with an ultradian frequency (Dement and Kleitman 1957), with usually one sleep phase comprising 3-5 cycles per night. Each cycle lasts about 80 - 120 min (e.g. Brezinová 1974, Brandenberger et al. 2001) and is subdivided

into sleep episodes of non-rapid eye movement (NREM) and rapid eye movement (REM) sleep. Based on amplitude and duration of sleep electro-encephalo-gram (EEG) waves (Rechtschaffen and Kales 1968), visual scoring divides sleep EEG into discrete sleep stages. Each night humans pass through 4 sleep stages during NREM sleep, which typically starts with stage 1 (transition between wakefulness and sleep), followed by stage 2 (light sleep), stage 3 and 4 (deep sleep or slow wave sleep, SWS; Figure 4). Additionally sleep spindles and K-complexes (stage 2; Fig.4) or vertex sharp transients (stage 1) are also typical events during NREM sleep. Thus, NREM sleep includes a variably synchronous cortical EEG, low muscle tones and reduced psychological activity (Carskadon MA 1989).

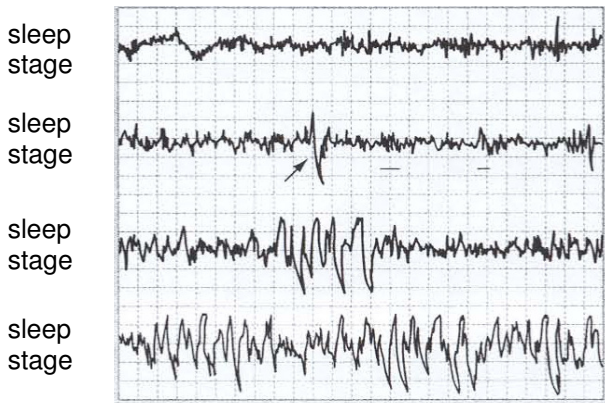


***Fig.4**: Typical patterns of EEG waves during NREM sleep; the deeper sleep, the slower and with higher voltage are the waves; arrow depicts a K-complex; underlines indicate sleep spindles; 2vertical squares represent 100mV, 4horizontal squares equals 5seconds.*
Modified from Carskadon 1989.

REM sleep is characterised by the appearance of a mixed frequency EEG, (similar to the waking EEG), low muscle tone in the electro-myogram (EMG) and rapid eye movements in the electro-oculargram (EOG). In terms of NREM-REM distribution across the night, it is well-established that the amount of SWS is the highest at the beginning of the night, whereas stage 2 and REM sleep occurrence increases in the second half (Carskadon MA 1989).

Spectral analysis, which decomposes the signal into single sine and/or cosine functions with specific amplitude and period, provides an accurate determination of frequency bands within each sleep stage. The sum of frequencies between 0.75 and 4.5 Hz characterises EEG slow wave activity (SWA), which acts as a functional index of increased homeostatic sleep pressure (Dijk et al. 1987, Dijk and Czeisler 1995). Furthermore, several frequency bands can be distinguished, including theta (4.75 to 7.75 Hz), alpha (8 to 12 Hz) and beta ranges (16 to 30 Hz).

Sleep–wake regulation

Borbely (Borbely 1982) first described the sleep-wake regulation, using a two-process model with two interacting processes: homeostatic and circadian processes, referred to as processes S and C, respectively. The quantitative version of this model elucidates that processes S + C are modulated by a single circadian process while the homeostatic one is determined by an upper and a lower threshold (Daan et al. 1984; Figure 5).

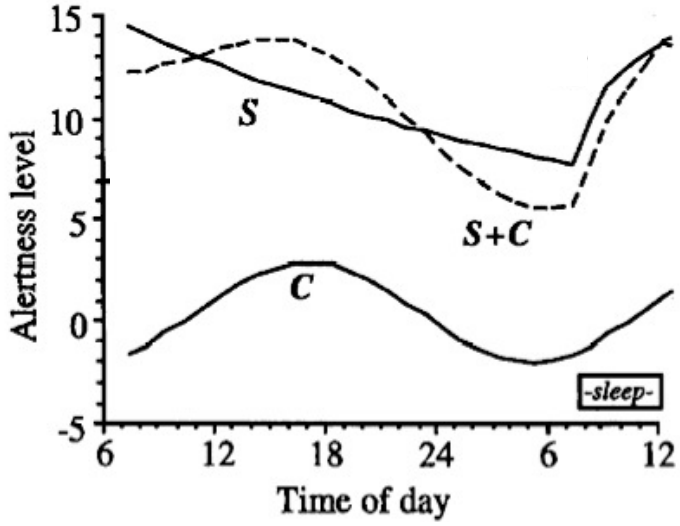


Fig. 5*: The 2 process model for sleep-wake regulation includes a homeostatic process (S), sleep pressure increases and alertness decreases in a near linear manner, until sleep occurs (here at 8 am). Additionally the model includes a circadian process (C) and the combined effect of both (S+C).*
Modified from Akerstedt et al. 2008.

Accordingly, the longer the wakefulness period, the more sleep pressure is built up (Borbely et al. 1981, Dijk et al. 1993). After an initial increase in sleep pressure, the schematic curve is characterized by an exponential development with elapsed time awake (Achermann et al. 1993). During the subsequent sleep episode, sleep pressure dissipates in an exponential fashion. Sleep pressure can be indexed by the amount of EEG SWA (Werth et al. 1997, Cajochen et al. 1999, Finelli et al. 2001), particularly in the beginning of the night, when sleep pressure is at its maximum level (Borbely et al. 1981, Dijk et al. 1993). Conversely, when sleep pressure is diminished due to naps during the day, SWA is reduced (Werth et al. 1996). Additionally, theta activity during wakefulness can act as an objective measure of enhanced sleep pressure (Cajochen et al. 1999, Finelli et al. 2000, Cajochen et al. 2001).

The neuroanatomical underpinnings for the sleep homeostatic process are still unknown. Evidence supports that local adenosine levels rise in definite cortical areas, predominant in the basal forebrain, during waking and decline during sleep (Huston et al. 1996, Strecker

et al. 2000, Murillo-Rodriguez et al. 2004). Local release of adenosine in this structure has been proposed as a signal for the homeostatic need for sleep (reviewed in Basheer et al. 2004). Further it has been suggested that adenosine promotes sleep by attenuating inhibitory input to the VLPO (Chamberlin et al. 2003). A further approach characterises wakefulness and sleep transition driven by the interplay between cell groups that cause arousal (monoaminergic system) and nuclei involved in sleep induction (e.g. VLPO). In that Saper et al. introduced the "flip-flop switch" model, whereby orexin neurons stabilises the system by activation of monoaminergic systems and inhibitory action on the VLPO (Saper et al. 2005; Fig. 6). Thereby a reduction of the orexinergic tone reduces wakefulness (McCarley 2007).

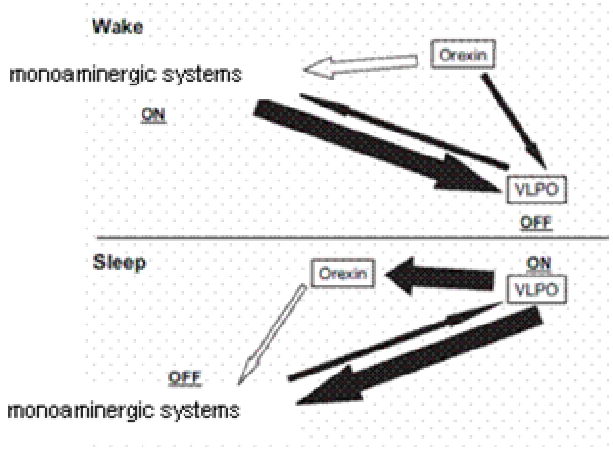


***Fig.6**: FlipFlop switch – regulation of wakefulness and sleep.*
Modified from Lu and Zee 2010.

Recent studies in rats suggest that adenosine interacts with the orexin system by reducing the activity of the orexin neurons (Thakkar et al. 2008). Since the orexin neurons seem to be under control of the SCN (Zhang et al. 2004) and adenosine reflects the homeostatic influence on sleep, the 2 process model of sleep seem to be confirmed.

The interaction of processes S and C is reflected by the compensation of the increase of homeostatic sleep pressure during wakefulness via the declining circadian sleep propensity (Daan et al. 1984, Achermann and Borbely 1994). A brain imaging study confirmed recently a direct interaction between sleep homeostasis and circadian rhythms in anterior hypothalamic areas (Schmidt et al. 2009). Further, the interaction of S+C may be seen in several cognitive processes, like in syntactic decision tasks (Rosenberg et al. 2009) temporal orientation (Späti et al. 2009) or cognitive performance tasks (reviewed in Blatter and Cajochen 2007).

Body Temperature

Human body temperature integrates the temperatures of the core (CBT) and the temperature of the shell (Aschoff and Wever 1958).

CBT combines the temperatures of abdominal, thoracic and cranial cavities, whereas the shell temperature is the sum of skin temperature, subcutaneous tissue and muscles. In contrast to the CBT, which is maintained by the brain at about 37°C, the shell temperature is predominantly modulated by the skin blood flow and environmental conditions (reviewed in Lim et al. 2008).

Thermoregulation

In humans the primary aim of thermoregulation is to sustain a stable core body temperature. This important regulation is generated and modulated in a homeostatic AND circadian manner.

A cluster of nerve cells in the hypothalamus is known to maintain CBT at a level of around 37°C (reviewed in Reilly and Waterhouse 2009). More precisely, animal studies suggest, that the homeostatic control of CBT is mediated by temperature sensitive neurons, located in the preoptic anterior hypothalamus (POAH; Satinoff 1978, Berner et al. 1999, Hori et al. 1999).

CBT is kept constant, when heat production and heat loss are balanced. However, it increases when rate of heat production exceeds rate of heat loss (Simon 1993). As a side product of all metabolic processes, heat is also a result of shivering, exercise and non-shivering thermogenesis. According to the second law of thermodynamics, heat transfer occurs from areas with high temperature to areas with low temperature. Thus, heat is dissipated from the core to the body surfaces to the environment at normal ambient temperature. The cardiovascular system plays an important role in heat transfer and distribution. Because of their large surface in relation to size, their skin capillaries and arteriovenous anastomoses (AVA), the distal skin regions (extremities) provide the best heat exchange. Whereas proximal skin regions (trunk) are less effective with regard to heat loss, as they do not develop AVA (reviewed in Kräuchi 2007a).

A still remaining question is how CBT is maintained, and what the mechanisms underneath. Several hypotheses, which address this question, are favoured, but neither hypothesis has been validated unequivocally.

Firstly the set point theory applies a fixed reference signal with which the actual CBT is compared (Bligh 2006). If CBT is above the reference temperature, heat fighting

responses (e.g. vasodilatation, preference of cold environment, sweating) get activated. Conversely, cold fighting responses (e.g., skin vasoconstriction, piloerection, increased thermogenesis, shivering, preference of warm environment) will be initiated if CBT falls below the reference temperature (Briese 1998; Figure 7).

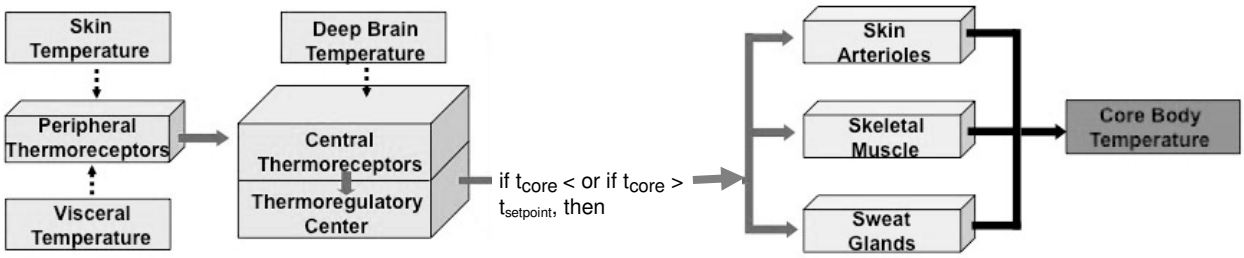


***Fig.7**: Set point theory displayed in a cartoon; input signals derive from peripheral thermoreceptors and receptors in the hypothalamus, signals are reported to the POAH, if temperature derivates from a certain set point heat-loss or heat gain responses are initiated.*
Modified from:_http://www.mdc-berlin.de/en/ research/research_teams/temperature_detection_and _thermoregulation/Research/Research_ Project_2 /index.html.

The usefulness of the set-point theory is still a matter of debate (Mekjavic and Eiken 2006). The precise regulation of CBT is seen as energetically expensive and physiologically unnecessary, since it is thought that an immediate sweating and shivering response is not necessary as soon as a displacement of CBT is determined (Mekjavic and Eiken 2006).

Alternatively, slight fluctuations in CBT may be buffered by vasomotion (e.g. vasoconstriction or vasodilatation), whereas larger fluctuations trigger the autonomic response of either sweating or shivering. Therefore an inter-threshold zone has been proposed in which CBT is maintained (Mekjavic and Eiken 2006; Figure 8).

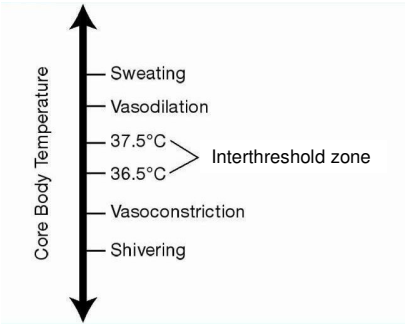


***Fig.8**: The inter-threshold zone is typically the range within core body temperature is maintained in humans; vasoconstriction and shivering are initiated, when core body temperature falls below and vasodilation and sweating, when temperatures rise above this range. Modified from Weant et al. 2010.*

The theory of reciprocal cross inhibition (RCI) presents another alternative.

RCI has been demonstrated to occur via 2 different pathways. In that, cold sensor to heat production and warm sensor to heat loss (HL) effector pathways have been described.

This dichotomy would be able to create the null-point temperature at which neither metabolic heat production nor evaporative heat loss effectors are active. In contrast to the set-point theory with a stable reference signal generator the RCI represents a model, in which an interplay of two variables with different response coefficients is promoted (Bligh 2006; Figure 9).

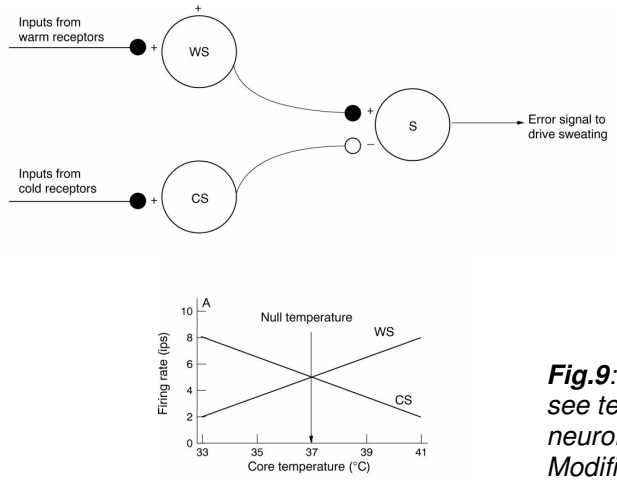


***Fig.9**: Theory of reciprocal cross inhibition; for details see text; warm sensitive neurons (WS), cold sensitive neurons (CS), integrating neuron (S).*
Modified from Gordon 2001.

In extreme situations, when ambient temperature is drastically decreased or increased, mammals are able to adapt to these temperatures. Short term adjustments are provided by activation of efferent autonomic outflow directed to the vasculature of the skin causing cutaneous vasodilatation which leads to an increase in skin blood flow, which in turn directly resulting in elevated shell temperature. Thus, heat dissipation to the environment and the rate of sweating are increased (Lim et al. 2008, Simon 1993). Taken together these mechanisms prevent the organism from a heat shock. The opposite can be seen during acute cold stress (reduced skin blood flow, diminished sweating), leading to a decrease of the shell temperature and thus, to a conservation of heat in the body (Lim et al. 2008, Simon 1993, Figure 10). Furthermore plasma expansion or contraction and changes in cardiac output are proposed strategies to acclimatize to short term changes in ambient temperature.

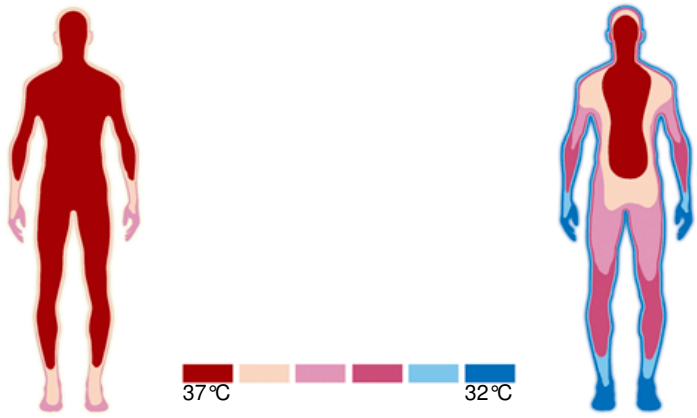


***Fig. 10**: The thermogram left shows the body temperature at warm ambient temperature (around 35°C). Similar distribution of heat like in the left thermogram occurs during sleep at normal ambient temperature. The thermogram right illustrates how heat is distributed in thermoneutral environments, if the subject is awake and standing.*
Modified from: http://www.bbc.co.uk/schools/gcsebitesize/science/images/body_temp_large.gif.

In summary, changes in the sweating or freezing response help the body to adapt to long term hot or cold temperature changes. The sweating/freezing response begins much later in a pre-acclimatised state compared to an acclimatised state. Thus, the sensitivity of this response is suggested to be changed (reviewed in Lambert and Dugas 2008). Because the heat - reacclimatisation was much faster after deacclimatisation, a molecular memory to acclimatisation was hypothesised (Tetievsky et al. 2008).

A rhythmic pattern of oral temperature with a maximum during the early evening and a minimum during the early morning has been described already in 1842 by Gierse (Gierse 1842). It was more than 100 years later that Aschoff investigated the mechanisms behind this physiological phenomenon (Aschoff and Wever 1958, Aschoff and Heise 1972). Importantly, this circadian time course is seen in stringent controlled constant routine settings, with and without sleep (Figure 11 e.g. Kräuchi et al. 1994, Kräuchi and Wirz-Justice 2001, Kräuchi et al. 2005), what is evidence for an endogenous circadian rhythmicity. Fluctuations in CBT within a 24 h day range between 0.6 and 0.9 °C with a broad maximum typically occurring from afternoon until evening while a trough has been identified in the morning at 4-6am (reviewed in Reilly and Waterhouse 2009). It is postulated, that rostral projections from the SCN are transferred to the POAH which may influence CBT in this circadian manner (Chou et al. 2002, Deurveilher and Semba 2003, Moore and Danchenko 2002, Saper et al. 2005).
Due to strong environmental (ambient temperature) and physiological influence (e.g. sleep) on skin temperature, a circadian pattern is only seen under controlled laboratory conditions (Figure 11; Kräuchi et al. 1994, Kräuchi 2006). The pattern of proximal skin temperature equals that of CBT; it rises in the morning and declines in the evening.

Contrary, distal skin temperature declines in the morning and rises in the evening (Kräuchi 2006).

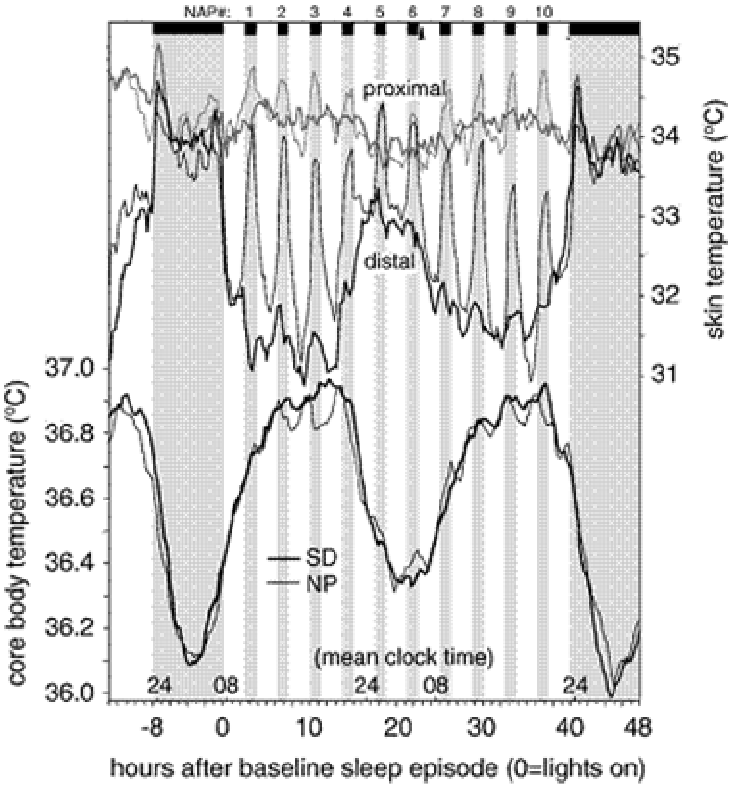


***Fig.11**: Time course of skin and core body temperature (N=8) recorded during constant routine protocols imbedded between 8h sleep (broad grey bars); SD = protocol with 40 hour prolonged wakefulness under 8lx (black curve); NP = nap protocol; ten cycles each of 75 min 0lx (thin grey bars) vs. 150 min 8lx (grey curve); proximal = weighted mean temperature of following skin regions: nfraclavicular, stomach, forehead and thigh; distal = mean temperature of inner wrists and surface of the medial calcanei. Modified from: Kräuchi 2007a.*

As described above, CBT is balanced by heat production and heat loss. The circadian rhythm of heat production is phase advanced compared to the circadian rhythm of heat loss (Aschoff et al. 1974, Aschoff 1982). Furthermore, heat loss dominates over a reduction in heat production in the evening, leading to a decrease of CBT. In the morning the opposite occurs, resulting in an increase of CBT (Aschoff and Heise 1972, Aschoff et al. 1974, Krauchi and Wirz-Justice 1994).

However, it is still highly debated how the circadian system interacts with the thermoregulatory system in detail (Cabanac et al. 1976, Aschoff 1983, Refinetti 1997).

Influence of thermoregulation on the sleep wake cycle

Sleep induction is closely coupled with the endogenous circadian rhythm of thermoregulatory processes. Increased distal and decreased proximal skin temperatures, as well as opening of arterio-venous shunts in distal skin regions has been demonstrated to promote heat loss (Krauchi and Wirz-Justice 1994, Kräuchi 2000). Interestingly, the onset of melatonin secretion occurs at the same time. Since it has been suggested that exogenous melatonin promotes distal vasodilatation (Kräuchi et al. 1997b, Kräuchi et al. 2000), one might assume that endogenous melatonin might likewise reduce vascular tone.

Additionally, haemodynamic changes associated with lying down result in increased proximal and distal skin temperatures, which in turn anticipate heat loss (Kräuchi et al. 1997b). Thus, it is suggested that distal vasodilatation and therefore heat loss, is associated with sleepiness and the rapid onset of sleep (Kräuchi et al. 1999a, Kräuchi et al. 1999b, Kräuchi 2000, Fronczek et al. 2006). Furthermore, sleep and wakefulness are related to thermoregulation, as clearly demonstrated during the "wake maintenance zone", whereby distal skin temperature is lowest - most vasoconstricted compared to other time episodes. At the same time CBT is high and the inner heat conductance reduced (reviewed in Kräuchi 2007a).

Taken together, this suggests an interrelationship of the thermoregulatory and the sleep-wake regulatory system. Animal experiments have conveyed more evidence for this assumption. In POAH lesion studies it has been shown that this area is not only responsible for homeostatic temperature regulation, but also directly involved in the regulation of sleep and wakefulness (reviewed in Kumar 2004).

Heart rate

Heart rate is influenced by intrinsic cardiac function, the interplay of sympathetic and vagal nerves at the sinoatrial node of the heart and by terms of circadian modulation.

Heart rate regulation by the autonomic nervous system

The autonomic nervous system (ANS), consisting of the sympathetic and parasympathetic branch, innervates mainly smooth muscle cells that are not under voluntary control (e.g. heart, lungs, blood vessels, etc.).

The sympathetic neurons leave the spinal cord at the thoracolumbar region. For example, sympathetic innervation of the heart is associated with the intermediolateral column of the spinal cord. In contrast to the parasympathetic branch, sympathetic nerves are characterized by shorter preganglionic neurons. After reaching the ganglion, postganglionic sympathetic neurons project directly to the target organ (e.g. cardiac smooth muscle; Figure 12).

The 10th cranial nerve is called the vagus nerve and innervates mainly the heart. This nerve, together with the 3rd, 7th and 9th cranial nerves, is part of the parasympathetic nervous system. They have longer preganglionic and shorter postganglionic neurons (Wagner&Silber 2004, Figure 12).

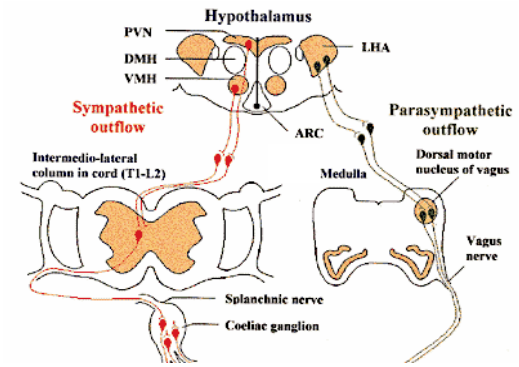


***Fig.12**: Neural pathway of sympathetic and parasympathetic branches;*
PVN = paraventricular nucleus; DMH = dorsomedial hypothalamus; VMH = ventromedial hypothalamus; LHA = lateral hypothalamic area.
Modified from King and Williams 1998.

The sinoatrial node (SA) in the right atrium in close proximity to the vena cava superior is the main pacemaker of the heart. Excitation of the SA node can be transmitted to both atria causing atrial contraction. Subsequently transduction of action potentials to the atrio-ventricular node (AV), along the bundle of His, bundle branches and fast conducting Purkinje fibre leading to concomitant excitation of different regions of the ventricles. Further excitation is transferred by the musculature of the ventricle (Figure 13).

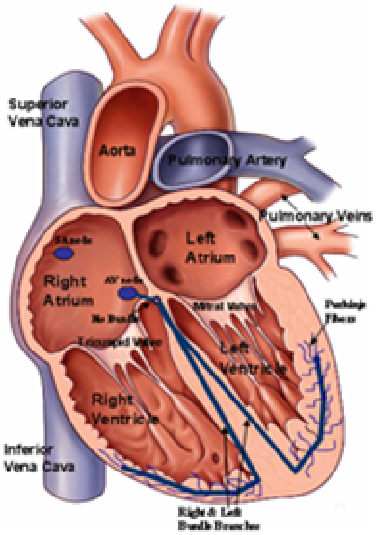


***Fig.13**: Anatomical display of the heart; SA = sino-atrial node; AV = atrio-ventricular node*
Source: http://www.washingtonhra.com/2.html.

Whereas the resting membrane potential in cardiac working cells is mainly caused by the equilibrium potential for K^+ ions, the membrane potential of cardiac pacemaker cells is never constant. It declines spontaneously (known as pacemaker potential) and if a certain threshold is reached an action potential is triggered, due to depolarisation by influx of Na^+ ions. Additionally slow influx of Ca^{2+} ions is responsible for a slower depolarisation, than in cardiac working cells. Afterwards the repolarisation is reached by inactivation of the slow Ca^{2+}-channels and increased K^+ ions outflux. However, the slope of the pacemaker potential is what determines the timing of the next action potential and thus, the intrinsic firing rate of the cell and the next heartbeat. The pacemaker potential slope is generated by reduced permeability to K^+ ions of the plasma membranes and by an inward pacemaker

current carried by Na^+ ions and to a later phase by Ca^{2+}ions (Levick 2000, Paulev 1999 - 2000).

As a result of the innervation of the sinoatrial node pathway by the ANS, heart rate either increases or decreases. For example, vagal activation via the neurotransmitter acetylcholine causes a slower heart rate, which is known as a negative chronotrope effect (Figure 14). Thereby acetylcholine activates the muscarinic acetylcholine receptors, resulting in the activation of G-protein-coupled K^+ channels (Mark and Herlitze 2000). Consequently this leads to hyperpolarisation of the membrane potential (decrease of the slope of the pacemaker potential) and thus, slowing of the heart rate. Conversely, the sympathetic release of adrenaline and noradrenaline has a positive chronotrope effect, thus increasing heart rate as a result (Fig. 14). This effect is mediated by an increased slope of the pacemaker potential caused by an increase of the inward current carried by Na^+ and Ca^{2+} ions.

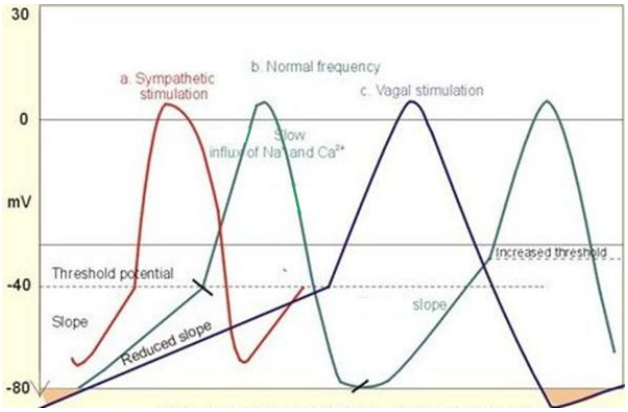


***Fig. 14**: Cardiac pacemaker cell potentials: a: sympathetic stimulation; b. normal heart rate; c. vagal stimulation. Modified from Paulev 1999 - 2000.*

Importantly, the differential responses of neurotransmitters at the heart are related to the presence of various receptors and receptor subtypes. Binding of adrenaline and noradrenalin to α1-adrenergic receptors will cause a vasoconstrictor response in resistance vessels, while binding to α2-adrenergic receptors will evoke vasorelaxation. Indeed, these neurotransmitters can alternatively act on β1-adrenergic receptors, leading to heart muscle contraction and accelerated heart rate and β2-adrenergic receptors, leading to vasodilatation of coronary blood vessels. Vagal induced release of acetylcholine might bind to muscarinic receptors e.g. M2 leading to cardiac deceleration and reduced myocardial contractility (Figure 15).

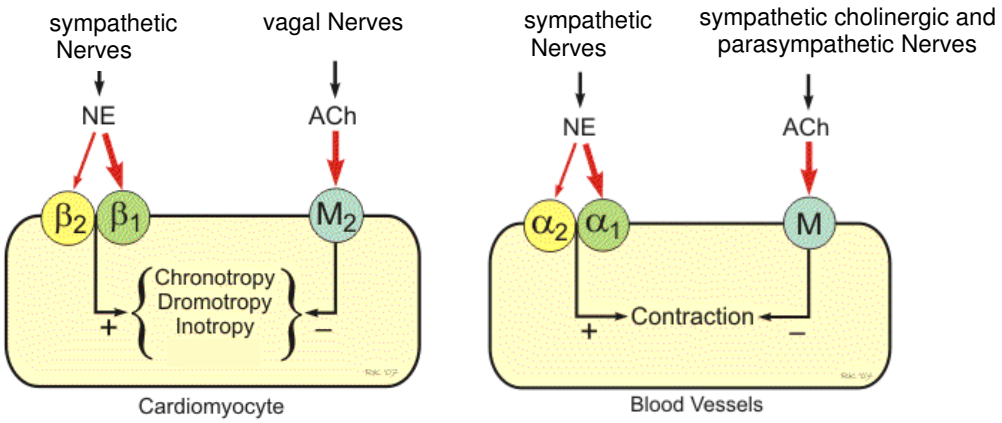


***Fig.15**: Action of the autonomic nerves on its receptors; NE = norepinephrine (= noradrenaline); ACh = acetyl choline; M = muscarinic.*
Modified from www.cvphysiology.com/Blood%20Pressure/BP010b.htm.

Beneath chronotrope influences to the heart, dromotrope and inotrope effects have been investigated on the isolated heart. In that, sympathicomimetica and thus, sympathetic activation is leading not only to a shortened AV transition (dromotrope), but also to an increase of the strength of muscular contraction (inotrope). Activation of the vagus evokes the opposite effect.

Vagal control of the heart occurs on a beat-by-beat control. It has been postulated that parasympathetic activation of the heart evokes its responses within only 50-100ms, mostly due to relatively short postganglionic neurons, and because of very immediate inactivation of the neurotransmitter. Contrary, the sympathetic nerves have been shown to exert slow cardiac responses, and thus, play a rather minor role in the short term control of the heart (Koeppen and Stanton 2010).

Some inconsistencies have been postulated in the vagal control of the heart, thus the so called "polyvagal theory" has been developed (Porges 2009). In that, a morphological division of the vagus into a myelinated and a phylogenetically older unmyelinated branch has been conducted. Myelinated vagal efferents have been shown to originate in the nucleus ambiguus (NA), unmyelinated vagal neurons on the other hand, arise from the dorsal motor nucleus (DMN). While the DMN branch is linked to primitive survival strategies (e.g. faigning death), the NA branch, only found in mammals, serves to promote calm behavioural states by e.g. inhibiting sympathetic influences to the heart. Within the theory, the functional organization of the autonomic subsystems is ordered phylogenetically hierarchical. The newest neural structures (myelinated NA branch) respond first, only falling back on older structures when a given response strategy fails (Porges 1995, Porges et al. 1999, Porges 2001, Porges 2003). The theory is widely accepted in the field of psychopathology (for further reading e.g. Beauchaine et al. 2007).

A clear day-night rhythm in resting heart rate has been demonstrated in rats, which occurs independent of locomotor influences. However, after SCN lesion, those rats showed an absence of circadian rhythm of heart rate (Scheer et al. 2001). Further evidence for circadian control of the heart rate reveal studies including genetic mouse models. Modified circadian clock functions resulted in abnormalities in the diurnal rhythm of heart rate (Curtis et al. 2007). Additionally, constant routine studies provide further evidence of the impact of the SCN on the heart. These studies reveal that heart rate responses in humans follow an endogenous circadian rhythm. More precisely, heart rates were higher during the day compared to the subjective night without sleep (Kerkhof et al. 1998, Krauchi and Wirz-Justice 1994, Scheer et al. 1999, Kräuchi et al. 1994, Hu et al. 2004, Viola et al. 2002). Results from animal studies indicate a multisynaptic pathway from the SCN to the heart. In detail, projections arising from the SCN to the PVN have been shown to function as a relay station. Further single neurons project to both, the adrenal cortex and the stellate ganglion, the main sympathetic ganglion, innervating the heart (Jansen et al. 1995, Scheer et al. 2001).

Peripheral clocks were recently discovered in human cardiomyocytes (Leibetseder et al. 2009). In animal models these clocks have shown to influence multiple myocardial processes, like the myocardial gene expression and contractile function in a temporal-dependent way. Thus it is suggested that the cardiomyocyte circadian clock regulates heart rate and contractility (summarised in Durgan and Young 2010).

Heart rate variability

Heart rate variability (HRV) describes the deviation over time of the period between consecutive heartbeats. The organised variability of the heart seems to mirror the adaptation to changing circumstances. Heart rate variability analysis is widely used as a parameter of the autonomic neural control acting on the intrinsic rhythm of the sino-atrial node of the heart, thereby providing valuable information about the balance between the sympathetic and vagal branch of the autonomic nervous system.

An example for a naturally occurring variability of HR is the respiratory sinus arrhythmia (RSA). In that, mainly under vagal control, inspiration elicits an increase in heart rate, while cardiac deceleration occurs during expiration (Koeppen and Stanton 2010; Figure 16).

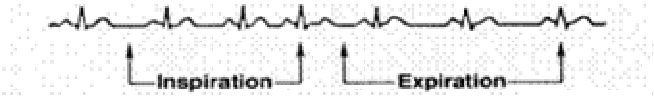


***Fig. 16**: ECG during inspiration and expiration.*
Modified from Cottingham et al. 1988.

Baroreflex activation is thought to be responsible for those decreases in heart rate during expiration. In detail, intrathoracic pressure and hence blood pressure is elevated during expiration. These pressure changes are sensed by the stretch-sensitive baroreceptors in the aortic arch and the carotid sinus. By adjusting their firing frequency, arterial baroreceptor supply the cardiorespiratory centres in the brainstem (i.e nucleus tractus solitarius) with important information. In response to increases in blood pressure the vagal branch of the nervous system will be activated, causing a reduction in heart rate (Koeppen and Stanton 2010). During inspiration the Bainbridge reflex is responsible for an increase in heart rate as follows: due to tension of the thorax, a falling intrathoracic pressure supports the return of venous blood to the heart, registered by stretch-sensitive receptors. Hence it results in cardio vagal inhibition and decreased heart rate.
It seems likely that the respiratory system is not only under vagal control. Indeed, circadian influences in the control of respiration have been proposed. Mammalian cells in the respiratory tract do have peripheral oscillators controlled by the rhythmic outflow of the SCN (Bando et al. 2007). Further, CR studies revealed a circadian pattern in some airway functions, like the forced expiratory volume (Spengler et al. 2000) or in respiratory chemoreflex characteristics (Stephenson et al. 2000). Following that, it can be assumed that the circadian effect on respiration is associated with 24 h fluctuation in the RSA amplitude.

Decreases in HRV have been shown to have diagnostic value for the incidence of cardiovascular disease and has been associated with increased mortality (Bigger et al. 1993, Tsuji et al. 1994, Dekker et al. 1997, Dekker et al. 2000, Huikuri et al. 2003, Stein et al. 2005, Guzzetti et al. 2005). Due to double pharmacologically blockade of vagal and sympathetic activity, it has been shown that HR is elevated compared to resting HR (Jose and Collison 1970), suggesting that under normal conditions the heart is under tonic inhibitory control of vagal activity. Thus, resting cardiac autonomic balance has a vagal dominance over sympathetic influences. As a result, high sympathetic tone is known to be a potent estimate of poor survival, whereas vagal activation is to some extent associated with cardio-protection (Cohn and Rector 1988).

As HRV can be non-invasively estimated by established calculations derived from ECG measurements, R-waves of the ECG indicate the spread of excitation over both ventricles and are easy to detect (Reilly and Antoni 1992). Usually the distance between R-waves (RR-interval, synonym: interbeat interval = IBI or NN-interval) are taken for analyses. The following paragraphs will describe the most common methods for the evaluation and visualisation of HRV.

Time domain method

Time domain measures are typically calculated by statistical analyses. They describe the dispersion of the sinus node cycle length around its mean (Zaza and Lombardi 2001). To analyse the global variance of the IBI-time series, standard deviation of all normal N-N intervals (SDNN) is calculated. If SDNN is calculated for a short time window (usually between 3 and 5 minutes), the values represent the short term variability due to rapid changes in heart rate (related to e.g. respiration). SDNN values over more than 5min reflect long term variability and provide information about slow fluctuations likely related to e.g. thermoregulation or the baroreflex. Another index of predominantly vagal activation is the proportion of successive R-R intervals that differ more than 50ms, measured as a percentage of the total number of the analysed ECG time window (pNN50%). Similar information derives from the calculation of the root mean square of successive differences of intervals (rMSSD).

Geometrical method

Geometrical measures have identified non-linear components of HRV in healthy humans (Braun et al. 1998, Lerma et al. 2003). The Poincaré plot is an example for a technique to visualise beat- to- beat variations and mirrors the non-linearity of signals. In this plot, the RR-interval is plotted as a function of the previous RR-interval (Kamen et al. 1996). A heart that beats continuously with the same frequency would be presented as a single dot in the plot. Due to the variability of the heart rate between consecutive heart beats a cloud appears in the plot, visualising short- and long-term cardiac variations of the data (reviewed in Lerma et al. 2003; Figure 17). The most meaningful parameters extractable from the 2-D plot are the extension and dispersion of each dot around the x-y-axis bisecting line. The dispersion of the cloud of points around the width along the bisecting line (SD1) is used as an indicator for short term HRV and the dispersion around the length along the bisecting line (SD2) is associated with long term HRV (Brennan et al. 2001).

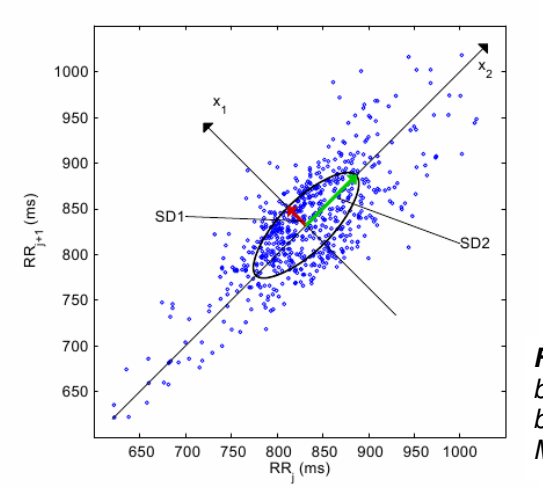


***Fig.17**: Poincaré plot; SD1 = width of the cloud along the bisecting line (x2); SD2 = length of the cloud along the bisecting line (x2).*
Modified from Kubios HRV version 2.0 Users guide.

Moreover, a correlation has been identified between SD2 and the global variance of the data (Guzik et al. 2007). On the other hand, SD1 is associated with the vagal part of the ANS by its correlation and mathematical equivalence with rMSSD (Kamen et al. 1996, Brennan et al. 2001). The ratio between SD2 and SD1 reflects the sympathovagal balance (Guzik et al. 2007). The extracted parameters SD1 and SD2 are mathematically indications of linear measures of HRV (Brennan et al. 2001), whereas it was shown, that the SD1/SD2 ratio detects the non-linear information of the HRV (Lerma et al. 2003).

Frequency domain method

Time series of IBI are the sum of several periodicities. To evaluate them, a Fast Fourier Transformation (FFT) of the IBI's is used. A Fourier Transformation describes a time-domain waveform in terms of frequency domain magnitude and phase (Ramirez 1985) by decomposing them into sinusoids and the FFT is an algorithm for evaluating the Fourier transform of sampled and digitized waveforms. The FFT produces a discrete frequency spectrum by calculating the overall power density per frequency line. The resulting power spectrum mirrors the magnitude of variability as a function of frequency.

A basic requirement is a stationary signal. Due to the non-stationary IBI waveform an equidistant resampling of short time windows (e.g. 0.0625 s = 16 Hz) has to occur. In this way a quasi-stationary signal can be obtained. To gain very slow waves e.g. the very low frequency band (VLF; 0.0033-0.04 Hz) or ultra-low frequency band (<0.0033 Hz), the length of the time window for the IBI's has to be set longer than the lowest frequency threshold, because the time window determines the slowest detectable wave and thus, the resolution. After the transformation leakage might occur. This can be controlled by "windowing". A data window fits the data or at least modifies them to a better form (Ramirez 1985). There are several data windows. The most common ones related to

evaluation of frequency domain of IBI's are the Hamming, Hann, triangular (Task Force 1996) or the Parzen window.

Vagal modulation of the heart is reflected by faster waves in the range of 0.15 Hz to 0.4 Hz in the high frequency band. The sum of frequencies in the range from 0.04 Hz to 0.15 Hz are summarised to the low frequency band. This band reflects slow waves derived from sympathetical and vagal modulation of the heart (Task Force 1996; Figure 18).

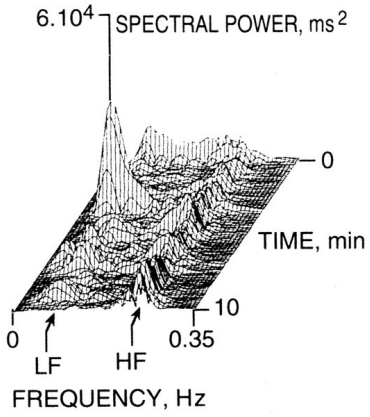


***Fig.18**: Spectral power plot of 10minutes duration; subject was in supine position and breathing at 0.25Hz; LF = low frequency; HF = high frequency.*
Modified from Jasson et al. 1997.

To refer to the sympathovagal balance, the ratio of LF band and HF band (LF/HF ratio) or the normalised spectral HRV measures low frequency (LFnu= LF/(LF+HF)) and high frequency (HFnu = HF/(LF+HF) are calculated. Each of the normalised spectral variables can be linear transformed to the other. As a consequence the information content of LFnu and HFnu is the same (Burr 2007).

Heart rate, heart rate variability and sleep

Sleep has an impact on cardiovascular functions. Day-night variations of heart rate were observed already in the 17th century (for further reading of historical issues on observations of rhythmic cardiovascular functions see: Lemmer 2009). The measured difference in heart rate between day- and night time was about 10bpm (e.g. Richardson 1971, Viola et al. 2002). Since the discovery of NREM and REM (Aserinsky and Kleitman 1953), research on the relationship between sleep stage and output of the heart has been carried out. Changes in ECG can begin prior to a sleep stage change (Bonnet and Arand 1998, Vasil'ev and Uryvaev 2006). In sleep stage 2, especially during arousal elicited K-complexes, the sympathetic nerve activity is increased (Somers et al. 1993). Slow wave sleep is related to the maximal reduction of respiratory rate (Murali et al. 2003) together with a decrease in HR. The HF component is high during NREM sleep (Berlad et al. 1993, Bonnet and Arand 1997, Ako et al. 2003, Abdullah et al. 2009). Thus, it is concluded that

during NREM sleep the vagal branch of the ANS is activated. Additionally NREM sleep is known to have lower heart rate variability than REM sleep (Brandenberger et al. 2001). During REM sleep, HR and respiratory rate changes rapidly. The LF component is high what is related to an increased sympathetic influence together with a withdrawal of the vagal compartment. This fact is confirmed by measurement of the frequency of the sympathetic burst, revealing an increased burst frequency during REM sleep (Somers et al. 1993).

Sleep stages are characterised by different EEG waves that appear to be coupled to ECG parameter. The LF/HF ratio seems to be coupled to the EEG mean frequency (Otzenberger et al. 1997). Further, delta EEG seems to be inversely correlated with LFnu and LF/HF and positive correlated with HFnu (Brandenberger et al. 2001, Abdullah et al. 2009) - at least during the 1st to 3rd NREM cycle (Ako et al. 2003). From this data a common central mechanism underlying EEG and HRV has been suggested. Contrary, dissociation between slow wave activity and LFnu has been shown in a study investigating cardiac autonomic control in subjects having a different genotype in a specific clock gene (Viola et al. 2008). Thus, future controlled laboratory studies, with subjects screened for age, gender and genotype have to unravel this incoherence.

Particular introduction

The relationship between thermoregulation, cardiac control and the autonomic nervous system is evident in various physiological events.

Naturally, heat is dissipated from warm regions (core) to colder areas of the body (skin surface) at normal ambient temperature not only by conductive heat transfer (second law of thermodynamics), but also, and most efficiently, by convective heat transport via blood flow (Aschoff and Heise 1972). The factors that are known to effect blood flow are best described by Hagen-Poiseuille's Law:

$$\text{Blood Flow} = \frac{\Delta P \, r^4 \, \pi}{\eta \, L \, (8)}$$

ΔP = pressure difference
r = radius of the vessel
η = blood viscosity
L = vessel length

Briefly said, blood flow depends proportionally on the driving pressure and the radius of the vessels; and inversely proportional on the vessel length and blood viscosity. Interestingly, blood flow has also been shown to be influenced in a time dependent manner, with maximal rate during the late afternoon and night hours and minimal rate

during the morning hours (Smolander et al. 1993, Caspary et al. 1997, Yosipovitch et al. 2004).

However, the radius of the vessels is indirect proportional to the vascular resistance and modulated by the autonomic nervous system (reviewed in Kirkman and Sawdon 2010). The cardiovascular system is a closed system; thus, the total blood flow leaving and entering the heart will be the same. In other words blood flow is corresponding to the cardiac output (CO). CO is modulated by the autonomic nervous system. In detail, CO - that is the blood volume ejected by one ventricle within one minute – is proportional to stroke volume and heart rate. Thus, autonomic control on heart rate influences CO and consequently blood flow. Additionally stroke volume is itself dependent on 3 factors. First it is influenced by the volume of blood in the ventricles at the end of the diastole that is dependent on the return of venous blood from the body. Changes in position, intra-thoracic pressure, blood volume and the tone in the venous system are known to influence the return of venous blood. Second stroke volume is dependent on resistance to ventricular ejection caused by the resistance to flow in the systemic circulation. The level of this systemic vascular resistance is controlled by the sympathetic system which controls the arteriole muscle tone, and hence the diameter. And third stroke volume is dependent on the release of noradrenaline by sympathetic fibres. This causes an increase in the strength of myocardial contraction, thus, increasing stroke volume via an increase of the intracellular concentration of calcium in myocardial cells (Rogers 1999, Levick 2000). Microneurographical and spill-over studies revealed that sympathetic nerves running to the skin include different fibre types. Cutaneous sympathetic vasoconstrictor nerves are tonically active under thermoneutral environmental conditions (Pergola et al. 1994). Fluctuations in the activity of these nerves are responsible for minor variations in skin blood flow that occur during normal daily activities.

Further, heat loss can efficiently be increased via opening of arteriovenous anastomoses in distal skin regions which are innervated by sympathetic vasoconstrictor nerves (Hales 1985). Withdrawal of vasoconstrictor nerve activity occurs during external elevated CBT (hyperthermia) and hence is responsible for increases in skin blood flow and heat loss (reviewed in Charkoudian 2003).

Another link between human thermoregulation and cardiac control was already observed in 1951. Tanner revealed a relationship between CBT and resting HR, measured in 46 healthy young men (Tanner 1951). During mild hypothermia an increase in noradrenaline concentration has been observed. Due to this autonomic activation and hence

vasoconstriction a compensatory decrease in HR occurs (reviewed in Sessler 2009). A further interaction is seen during fever. Due to temperature sensitivity of the cardiac pacemaker rate, HR is increased by about 10 bpm if CBT increases about 1K (Levick 2000). Practical use of that observed correlation between CBT and HR can be found in cardiac surgeries. Cooling of the body reveals a decrease in HR and thus, in cerebral blood flow, leading to neuroprotection (e.g. Nathan et al. 2007). Problems occur in this context, since peri-operative hypothermia is leading to disease-causing cardiac outcomes (reviewed in Sessler 2009).

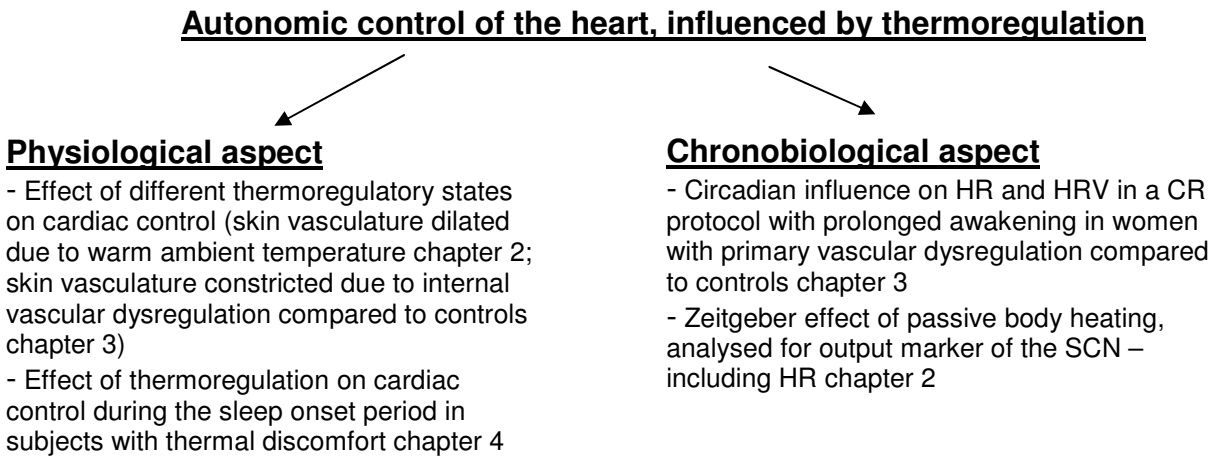
Interestingly thermoregulatory oscillations are thought to be of a non-linear character (Sayers 1973). Thus, they might just be seen in non-linear HRV assessments. Nevertheless, studies in the 70's report on a peak at around 0.03-0.04 Hz of spectral HRV analyses that is related to the thermoregulatory system (Kitney 1974, Rompelman et al. 1977). About 20 years later studies that induced a reduction of subjects' CBT confirmed an increase in the power of the VLF component of spectral HRV analyses (Fleisher et al. 1996). Additionally a blunted, but still evident peak in VLF has been investigated after acclimatisation to cold environment (Makinen et al. 2008). Another study with thermal skin stimulation at 0.1, 0.03, 0.02 and 0.01Hz revealed an increased 0.1 Hz periodic HRV (Lindqvist et al. 1989). Therefore the author suggests an induction by a central sympathetic activation of the vascular blood pressure control system. Furthermore cutaneous thermal, thus, hypothalamic stimulation, might lead to a decrease of the sensitivity of the vagal baroreceptor modulation of the HR (Lindqvist et al. 1990).

Gender seems to have an influence on HRV when the thermoregulatory state is changed. In detail men showed an increased peak in VLF power in a cold environment while VLF power of the women changed very little (Sollers et al. 2002).

All presented publications that investigated HRV under changed thermoregulatory states have a lack of certain information, like e.g. time of day or measures for both, skin and core body temperature in combination with HRV analyses. Thus, from a chronobiological and physiological point of view the interaction between autonomic cardiac control and thermoregulation is of high interest.

Objectives and structure of the thesis

The main focus of the present thesis is to broaden the basic knowledge about the interaction between autonomic cardiac control and thermoregulation, as illustrated below.



Thereby subject's thermoregulatory state is modified either by passive body heating (chapter 2) or is investigated in subjects with endogenous dysregulation of distal vessels, leading to thermal discomfort from cold extremities (chapter 3 and 4).

In chapter 2 both physiological and chronobiological aspects of passive body heating on HR and HRV will be addressed. Warm sensitive neurons and warm sensitive ion channels are located in the hypothalamic brain region (Van Someren 2003, Whitten et al. 2009). Thus an increase of skin temperature and CBT might affect distinct areas in the hypothalamus, like the SCN. If temperature is able to shift output rhythms of the SCN, it might be seen a shift in HR and/or HRV. Further, if temperature is sufficient to act and might be applied as zeitgeber, the acute implication of heat to the cardiovascular system is of great interest. Studies that determined heat exposure on HRV in young subjects found an increase of sympathetic and decrease of the vagal activity, in line with an increase of heart rate (Brenner et al. 1997, Crandall et al. 2000, Nagasawa et al. 2001, Kataoka and Yoshida 2005). In these studies the effects of passive body heating on HR and HRV might have been confound by a potential masking effect of exercise or food intake. In that, by application of a very controlled protocol in the study of chapter 2 the acute effects of passive body heating on cardiac control have been investigated.

Of note, most publications about HRV derive from studies in male subjects or from mixed gender groups. However, gender influences have been reported. In that, during sleep men elicit a withdrawal in vagal tone and an increase in ratio of LF/HF power compared to women. Further a vagal decrease during REM sleep in men compared to women was found (Elsenbruch et al. 1999, Valladares et al. 2008). The studies described in chapter

3&4 are conducted only in female subjects, what extends the previous knowledge of the interaction of cardiac and thermoregulatory control specifically for this group.

In chapter 3, HR and HRV analyses will be assessed in a group of women including a different thermoregulatory state (Vollenweider et al. 2008) and compared with the results derived from a control group of women. Is it possible to disclose changes in HR and/or HRV in a group with a chronically disturbed phase of entrainment? Although a relation of the rhythmic output of the SCN and the autonomic control of the heart is suggested, a circadian impact on the variables of HRV is still a matter of debate. Different controlled protocols (e.g. sleep vs. non sleep routine, constant routine prolonged awakening, forced desynchrony) and different measures for vagal and sympathetic activity (RSA, rMSSD, HF as vagal HRV measures; pre-ejection period, LF, LFnu as sympathetic HRV measures) reveal equivocal results (Burgess et al. 1997, Hilton et al. 2000, Viola et al. 2002, Vandewalle et al. 2007, Viola et al. 2008). The issue of a circadian component in HR and/or HRV is addressed in the third chapter, within a stringently controlled protocol with 40h awake. Frequency domain methods are used to draw conclusions about the vagal activity and the sympathovagal balance.

In chapter 4, HR and HRV will be analysed in young healthy women during the sleep initiation period. The impact of sleep itself on HR is known. A shift of the sleep phase to day times show that HR decreases about 10bpm as it has been reported to happen during night sleep. Any of the measured HRV indices failed to show differences between day time and night time sleep episodes (Viola et al. 2002). However, especially the sleep onset period is characterised by striking changes in heart rate, body temperature and EEG wave amplitude and frequency. Skin temperature, especially the gradient between distal and proximal skin temperature, is known to be an easily measurable variable for distal vasodilatation, which is autonomically controlled. The relationship between skin temperature and cardiac autonomic control during sleep initiation has not been yet investigated. To extend the timeframe from lights off to sleep onset and from vasoconstriction to vasodilatation data will derive from subjects that have thermal discomfort from cold extremities and difficulties initiating sleep. Furthermore, to our knowledge it has not been investigated, if changes in sleep, temperature or cardiac regulation occur after constant posture conditions without prolonged wakefulness. Thus, in chapter 4 the pattern of core body- and skin temperature, heart rate and its variability will

be elucidated during the sleep initiation period, examined on two subsequent nights in the laboratory.

In order to distinguish exogenous and endogenous rhythmicity all data derived from subjects for the thesis were gathered under exactly controlled conditions in the chronobiology laboratory.

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Chapter 2

Is an evening heat pulse a zeitgeber in humans?

Doreen Anders*[1], Britta Gompper*[1], Vera Knoblauch[1], Andreas Schötzau[2], Jörg Weik[1], Christian Cajochen[1], Anna Wirz-Justice[1] and Kurt Kräuchi[1]

[1]Centre for Chronobiology, Psychiatric Hospital of the University of Basel, Basel, Switzerland; [2]University Eye Clinic, Basel, Switzerland

*These authors contributed equally to this work

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Abstract

The claim that temperature is a weak synchronizing zeitgeber in homeotherms has only rarely been addressed in humans. We tested the phase shifting capacity of a thermal load in the evening in healthy young men during a stringent controlled 40-hour 'constant routine' protocol. A thirty minute warm bath in the evening induced a consistent 2-hour phase advance as measured by three different circadian marker rhythms (core body temperature, heart rate, and melatonin secretion) suggesting a zeitgeber effect of temperature on the circadian clock. To our knowledge, our study is the first to show that a heat pulse via warm bathing is a zeitgeber in humans, and provides evidence for considering heat as a non-photic zeitgeber. Further research is needed to identify the mechanisms.

Introduction

In contrast to poikilothermic organisms, homeotherms exhibit weak sensitivity to temperature as a synchronizing agent (zeitgeber) (11). Ambient temperature has the capacity - at least in some mammals - to influence the entrainment of activity rhythms (7-9, 21, 23, 32). However, zeitgeber studies of environmental temperature changes are rare in humans and insufficiently controlled to draw a final conclusion (35, 36). Homeotherms developed efficient physiological and behavioral mechanisms to protect the body core against environmental temperature changes, one of which incorporates the central biological clock located in the hypothalamic suprachiasmatic nuclei (SCN). Therefore it is quite possible that in larger species such as humans, larger temperature changes than those occurring during natural physiological conditions are required to notably affect the central clock.

In the last decade many *in vitro* studies have shown that temperature modifies central and peripheral circadian clocks. For example, experiments with mammalian SCN slices have demonstrated that the circadian rhythm of neuronal firing rate can be phase delayed or advanced with a heat load in the early and late subjective night, respectively (24, 25). It is primarily peripheral clocks which are highly sensitive to temperature changes in a physiological range (4).

When the human circadian system is shifted in mammals *in vivo,* e.g. by light administration, a change in the circadian phase of overt phase marker rhythms occurs. One of the best-studied rhythms is core body temperature (CBT) (28). Physiologically,

CBT reflects a major part of total body heat content. Therefore, a change in body heat content has to accompany the shift, and can result from changes in body heat loss, heat production and/or heat load. In previous studies, we have shown that evening administration of bright light acutely retains body heat content (5, 13), while melatonin acutely reduces it (13), and 24 hours later a phase delay and advance occur, respectively (13). Additionally, we have shown that an evening food pulse could elevate CBT and heart rate (HR) and induce a phase delay measured one day thereafter (14). These results suggest a causal relationship between the acute thermoregulatory effects of a zeitgeber administration and the capacity to induce a phase shift.

We thus hypothesized that body heat load at the beginning of the subjective night should increase CBT and hence induce a subsequent phase delay of the circadian system. In order to test this hypothesis we applied a warm bath for 30min in the evening which elevated CBT by about 1°C. The present study was planned to differentiate between an acute masking effect on circadian phase and a real phase delay of the circadian system, as measured 24 hours later by the circadian rhythms of CBT, HR and melatonin (MEL).

Methods

Subjects

During March to November 2007 eight male subjects (age: 23-29 y; BMI 20-23.5 kg/m^2) were recruited via internet advertisements at the Universities of Basel, Zürich and Freiburg (Germany) for the intervention group. We compared these data with data gathered in 8 male controls (age: 21-29 y, BMI 19.6–24.7 kg/m^2) who had previously carried out an identical 40-h constant routine (CR) without any intervention.

Volunteers had to complete screening questionnaires: a chronotype questionnaire as well as a specially developed questionnaire covering sleep habits, sleep quality, life habits, physical health and medical history. Exclusion criteria were extreme morning or evening types and poor sleep quality. Subjects had carried out no shift work within 3 months or a transatlantic flight within 1 month before the study commencement; they had no medication or drug abuse and were non-smokers. Subjects were given a medical examination to establish their physiological and psychological health. After screening, subjects underwent a polysomnographically (PSG) recorded night in the sleep laboratory to confirm adequate sleep (sleep efficiency ≥85%). The main purpose and risks of the study were explained to the subjects before they gave their written consent. Subjects could stop the experiment at any time. The experimental protocol was approved by the local

ethics committee for human studies and conformed to the declaration of Helsinki. All subjects completed the study without any complaints.

Study design

One week before the start of the laboratory protocol, subjects were screened for seven days. They were instructed to keep a regular sleep-wake schedule (sleep times within ± 30-min of their habitual sleep times, as indicated in the screening questionnaire; sleep duration: 7-9 h). During the screening week they were instructed to have not more than 200 mg caffeine/day, not more than one glass of alcohol/day and were restricted to < 5-h exercise/week. Compliance was checked by sleep logs and a wrist worn activity monitor (Actiwatch®-L, Cambridge Neurotechnology Ltd., United Kingdom). Habitual sleep time was calculated by averaging the lights off times during the screening week.

Participants underwent an adaptation and a baseline night followed by an adapted CR protocol (18) with 40-h prolonged wakefulness in near-supine position (not more than 45°). Subjects were allocated individually to a sound-attenuated chamber controlled for light (<8 lx at the angle of gaze) without any time cues. Ambient air temperature (22° C) and relative humidity (55%) were constant throughout. Isocaloric sandwiches were administered at hourly intervals and water was available ad libitum. The CR was followed a recovery night in the laboratory.

The intervention group underwent passive body heating (PBH) 14.83 hours after beginning the CR. For this, they were transported via a wheeled bed to a bathroom 10 min before immersion into a bathtub with 39°C warm water (head out) for 30 minutes. In order to reduce postural changes, a crane - available for hospital use - for lifting subjects into and out of the bathtub was utilized. Bath water temperature was monitored via a digital thermometer and a thermosensor in the water. Subjects wore dark sunglasses during the whole intervention procedure to assure the same dim light conditions as in the laboratory room. The participant changed clothes in lying body position before and after the bathing procedure.

Controls participated carried out an identical CR protocol but without this intervention (15). This approach using parallel groups is justified because it is highly unlikely that the transport and bathing procedure *per se* possess any potential circadian zeitgeber capacities.

Measurements

Thermometry: Rectal temperature was registered by a thermocouple (polyoxymethylene probe: 2-mm diameter, copper-constantan, accuracy: 0.01°C; Interstar, Cham, Switzerland; therm-device: Ahlborn, Holzkirchen, Germany) self-inserted 10 cm into the rectum, sampled every 30-s.

Salivary melatonin: For melatonin analyses saliva was collected (1–2 ml). The samples were immediately refrigerated at 5° C, centrifuged within 2 days and stored at –20°C. 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 pg/ml; Bühlmann Laboratories, Schönenbuch, Switzerland; (34).

Electrocardiogram (ECG): Standard Ag-AgCl surface electrodes were placed on V2 and V5 (modified precordial lead). ECG was recorded with the VITAPORT ambulatory system (Vitaport-3 digital recorder, TEMEC Instruments B.V., Kerkrade, the Netherlands) with a sampling rate of 256 Hz. The raw signals were stored online on a Flash RAM card (Viking) and later downloaded off-line to a PC hard drive.

Data analysis and statistics

All raw data recorded during the CR protocol were inspected visually for plausibility. Affected data e.g. due to rectal probe slips, were deleted. Missing data were replaced by a linear interpolation procedure. Data of CBT, HR and MEL were calculated as deviations from the 24-h mean values. The values at the time of the acute effect were not used for calculating the mean. Dim-light melatonin onset (DLMO) was determined with linear interpolation for each melatonin profile, when the threshold of the mean of 3 consecutive low daytime values plus twice the standard deviation of these points were exceeded and remained above the threshold for at least 1 hour. For heart rate measures a computerized system (System Hofstetter, SHS Allschwil) was used to analyze the signals and detect the length of all R-R intervals in 5-min episodes throughout the whole CR protocol – each output was checked for plausibility by visual inspection and all R-R peak durations < 0.3-s and >1.8-s were eliminated. If the noise was low, this time duration was taken for further analyses. HR was calculated as 1/Median*60 for all 5-min sequences. Core body temperature (CBT) and HR data were averaged in 15-min bins for each subject. Data of the first 2.5 h of the CR were excluded from analysis, to eliminate any residual effects of prior sleep on the tested variables.

The time course of CBT and HR were analyzed by a nonlinear mixed effects model. Data of the 20-h time segment starting 20 h after lights on, corresponding to 5.17 h after bath immersion was taken for statistical analyses. The following model was used to fit circadian amplitude (A) and phase (ϕ), whereby:

$y(t) = C+A*\sin(2*\pi\ (t+\phi)/24h)$

y= CBT (°C) or HR (beats per min); before data analyses individual y (t) –values were adjusted to the averaged values within time segments BASELINE and POST-TREATMENT DAY

t = time (hours) after lights on

C= intercept

Nonlinear models were calculated using the package nlme (20) performed by R (V2.10.1; R Development Core Team 2008). ANOVA, MANOVA and regression analyses were performed using Statistica™ 6 for Windows (StatSoft Inc., Tulsa, USA) and the statistical package of Statview™ 5.0.1 (SAS® Institute Inc., Cary, USA). Huynh-Feldt (H-F) statistics were used to adjust the covariance matrix for violations of sphericity. H-F P values were based on corrected degrees of freedom, but the original degrees of freedom are reported. SigmaPlot® 11.0 for Windows (Systat Software, Inc., Chicago, USA) was used for graphics. Results are reported as means ± SE.

Results

Baseline

In order to test whether the time course before intervention did not differ between the intervention- and control group, 2-way ANOVAs for repeated measures were calculated for data of the 11-h time segment starting 2.5-h after lights on. Neither the time course of CBT and HR during baseline (CBT: GROUP: $F_{(1,14)} = 0.007$, $p = 0.93$; HR: GROUP: $F_{(1,14)} = 0.485$, $p = 0.5$), nor the DLMO (time after lights on: controls, 13.819 h ± 0.213 h vs. intervention group, 13.039 h ± 0.405 h; $df_{(1,14)}$, $p = 0.11$) differed statistically.

These data provide the required similar starting conditions with respect to circadian phase. In Figure 1 the rhythms of CBT, HR and MEL are shown for the intervention group and controls over the whole CR.

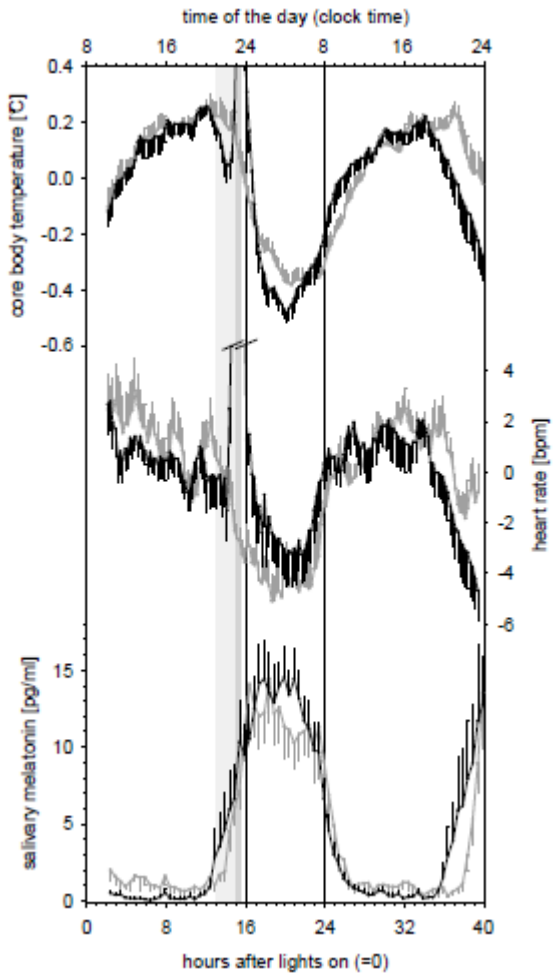

Figure 1 Time course (mean ± SE) of core body temperature (15-min bins), heart rate (15-min bins; both as deviations from the 24-h mean value) and salivary melatonin values (in half-hourly intervals) for the intervention group (black; N = 8) and controls (grey; N = 8) during a constant routine protocol with 40-h sustained wakefulness (data are plotted from 2.5 h to 40 h after lights on = 0). The intervention (warm bath, 39°C) is indicated by the grey bar, subjective night (where they would have slept) by the blank area between two vertical lines, and the time of masking effects due to preparing for the intervention by the light grey bar.

Acute effect

In comparison to the control group, PBH significantly increased CBT and HR in the intervention group (see Table 1).

In both CBT and HR PBH induced significant differences between the control- and intervention group. In order to localize the differences between the 2 groups, linear contrasts were calculated using Duncan's multiple range test. Significant differences in GROUP were found 15.125-h after lights on until 16.375-h after lights on for CBT and 14.625-h until 16.375-h after lights on for HR. CBT and HR increased maximally by 1.01°C and 32.98 bpm, respectively (unpaired t-tests: $p < 0.05$).

Table 1:

Variable	Group		Time		Time x Group	
CBT	$F_{1,14} = 6.015$	$p = 0.02$*	$F_{17,238} = 61.931$	$p < 0.0001$*	$F_{17,238} = 29.520$	$p < 0.0001$*
HR	$F_{1,14} = 44.227$	$p < 0.0001$*	$F_{17,238} = 45.056$	$p < 0.0001$*	$F_{17,238} = 48.984$	$p < 0.0001$*

ANOVA for repeated measures were calculated for CBT and HR for the ACUTE-effect, meaning the time segment between 13.5-h after lights on and 18-h after lights on (=time segment including data before intervention [1-h], during [0.5-h] and acute after-effects [3-h]).

Post-treatment day

Data of the control group were used for internal validation of the phase estimation method. Estimated phases of the 20-h post-treatment day time segment were compared with those of the 37.5-h CR episode starting 2.5 h after lights on. Linear regression analysis revealed statistical significance (POST-TREATMENT DAY vs. CR: CBT, $r=0.990$, $p<0.05$; HR, $r=0.756$, $p<0.03$; $N=8$), indicating sufficient accuracy of the phase estimates using the short 20-h post-treatment day segment.

An unpaired t-test was used to elucidate differences between the intervention- and control group (DLMO**:** controls: 14.175 h ± 0.263 h vs. intervention group: 12.719 h ± 0.471 h; $df_{(1,14)}$, $p < 0.05$).

In order to compare the extracted circadian phase estimates of CBT, HR and MEL during the post-treatment day, a MANOVA for repeated measures was calculated (GROUP: $F_{(1,14)} = 7.887$, $p < 0.02$; PHASE MARKER: $F_{(2,13)} = 147.3$, $p < 0.001$; GROUP x PHASE MARKER: $F_{(2,13)} = 1.112$, n.s.). In comparison to controls, the intervention group revealed a significant phase advance (pooled phase advance: 2.1 h) without any influence of the type of phase marker (GROUP x PHASE MARKER, n.s.), indicating a phase advance of the entire circadian system (see Figure 2; separate analysis of each phase marker revealed also significant differences; all $p < 0.05$). This conclusion is supported by significant inter-correlations between the phase markers (CBT vs. HR, $r = 0.732$, $p < 0.05$; CBT vs, MEL, $r = 0.744$, $p < 0.05$; HR vs, MEL, $r = 0.791$, $p < 0.05$; $N = 16$).

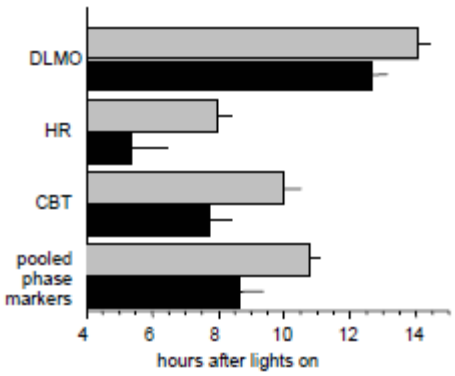

Figure 2 Circadian phase (mean ± SE) of core body temperature (CBT), heart rate (HR), dim-light melatonin onset (DLMO), and of the pooled phase marker -measure (averaged circadian phase of CBT, HR and MEL) for the intervention group (black) and controls (grey) extracted from the post-intervention period (20-40 h after lights on). Comparison between groups revealed significant differences in all measures.

Discussion

Our study has shown that a warm bath in the evening induced a significant phase advance by 2 hours in CBT, HR, and MEL measured the day after the intervention. This is a large phase shift (a single application of melatonin or light induces a 0.5 - 1h shift), and is in contrast to what we expected. Several factors might have contributed, but the following can be ruled out. First of all, the main zeitgeber in mammals, light, which was stringently controlled by dim light settings in the laboratory and sunglasses during the bathing procedure, would have led to a phase delay and not to a phase advance after evening administration. Further, social contact as a non-photic zeitgeber can also be excluded. All participants had the same regular contact with study helpers throughout the entire 40-h study. Additionally, the activities involved in the bathing procedure are too small to be considered “exercise”, which might induce a phase shift. Thus, how can we explain why the evening heat load phase advanced the circadian system rather than delaying it?

In a range of organisms the phase response curve (PRC) to temperature is similar to the PRC to light (1, 26), suggesting a phase delay of the SCN in the late evening after administration. This is in accord with the fact that both light and heat are emitted from the sun and support each other to phase entrain the organism’s circadian system. However, in nocturnal and diurnal species, non-photic zeitgebers with arousal-dependent properties, e.g. feeding, induce phase shifts in the opposite direction at the same circadian times (6). This leads us to suggest a different underlying mechanism for the effect of heat and light on the human circadian clock. Recently Refinetti showed an estimated exercise PRC in mammals, that is not similar in shape to the light PRC, confirming different PRC`s for photic and non-photic zeitgebers (21).

PBH for 30 minutes induced the expected increase in CBT and HR which lasted 3 to 4 hours. It is known that movements / posture changes can modify redistribution of the blood circulation and thus have an effect on skin and rectal temperature as well as on heart rate. However, movements and posture changes were kept to a minimum in this study by using the constant routine protocol throughout, a wheeled bed for transport from the laboratory bed to the bath and back, as well as a crane for lifting subjects into and out of the bathtub. Nevertheless, some postural changes due to preparation before PBH (head up during CR at 45° vs. during transport 0°; etc.) probably account for the acute differences in CBT and HR between the 2 groups just before the intervention. Furthermore, it can be assumed that PBH increases not only temperature in the abdominal tract and skin, but also in the brain (17, 19, 37). A warm stimulus registered by skin thermoreceptors (e.g. TRPV3) elicits brain activation via afferent C-fibers (reviewed in: 10, 27). Central thermoreceptors such as warm-sensitive ion channel receptors in the anterior hypothalamic brain region have been described, and warm-sensitive neurons are found in the SCN (33). Therefore, both peripheral and central thermoreceptors can potentially convey heat information to the SCN, but also the effect of a direct increase in hypothalamic temperature has to be taken into account.

On the post-treatment day 24 hours after the intervention CBT declined earlier. This might be due to a time memory for heat exposure, with the heat memory response down-regulating CBT in advance to avoid a potential recurrent heat stress. This explanation seems promising, because several studies have revealed that the CBT of rats and humans decline in advance even if the heat pulse is no longer given (16, 29, 30). Such a time memory seems to imply a cytoprotective network pathway (31). In particular, the SCN is the dominant brain area where a heat memory is built and maintained (16). However, studies providing evidence for a heat memory usually applied daily heat pulses over several days. Our findings suggest that a single heat pulse for 30 minutes is sufficient to build a heat memory response in humans.

Recent studies of the neurophysiology and molecular biology of circadian rhythmicity allows a better understanding how the central master clock (SCN) synchronizes (entrains) peripheral clocks. It has been stated that temperature affects circadian clock gene expression and its negative feedback loop (22). The integral clock components *mPer1 and/or mPer2* are likely to be involved in the synchronization of circadian clocks (12). The accumulation of these clock components is highly dependent on heat shock factor 1 and components of the heat shock response pathway. Primarily it is peripheral clocks that are

highly sensitive to environmental temperature (3), (4). However, when communication between cells within the SCN is blocked the tissue exhibits also temperature sensitivity (4). These studies would favor the assumption that only peripheral clocks are shifted by the evening PBH without a contribution from the SCN. If this is correct we have to assume that a phase advance of the peripheral clocks by the evening PBH overrides the master clock, such as observed for the food-entrainable oscillator (FEO) (2). An alternative hypothesis could be that a CBT increase by 1°C in humans leads not only to a phase shifts in peripheral clocks but also in central clocks in the SCN. For this hypothesis we have to assume that the CBT elevation is sufficient to disturb communication between cells in the SCN which seems to be necessary to induce a circadian phase shift in the master clock (4).

Limitations

Zeitgebers in nature are often repetitive daily signals. They are more effective when applied more than once and they are intensity-dependent. We can consider this study as just the beginning of efforts to evaluate a PRC with different heat pulse intensities. Measurements at the molecular level (e.g. mPer2) within the same protocol could reveal more about underlying mechanisms.

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Chapter 3

HEART-RATE VARIABILITY IN WOMEN DURING 40-HOUR PROLONGED WAKEFULNESS

Doreen Anders[1], Stephanie Vollenweider[1], Judith Cann[1], Marcel Hofstetter[1], Josef Flammer[2], Selim Orgül[2], and Kurt Kräuchi[1]

[1]Thermophysiological Chronobiology, Centre for Chronobiology, Psychiatric Hospital of the University of Basel, Basel, Switzerland
[2]University Eye Clinic, Basel, Switzerland

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Abstract

Heart-rate variability patterns of 18 women during a 40-h constant routine of prolonged wakefulness under controlled laboratory conditions were analyzed. The authors tested the circadian timing of the autonomic nervous system and the relationship between the sympathetic and vagal branches in women with both a functional disorder of vascular regulation (main symptom: cold hands and feet) and prolonged sleep onset and controls without these symptoms. Spectral analysis of R-R intervals during paced breathing episodes revealed significantly lower power values in the high-frequency band (HF; 0.15–0.4 Hz) but not in the low-frequency band (LF; 0.04–0.15 Hz), leading to a significantly elevated LF/HF ratio in the former group. A significant circadian rhythm in LF power and heart rate occurred in both groups, and a significant correlation was found between sleepiness and sympathovagal balance ($r = .53$, $p < .05$). These findings indicate not only an autonomic imbalance in the first group compared with controls, but also two strategies of the autonomic nervous system to fight against fatigue in women. One implies circadian control and the other homeostatic control, and both are reflected by the LF/HF ratio.

Introduction

Women with symptoms of both primary vascular dysregulation (PVD) and difficulties initiating sleep (DIS) have a mismatched internal phase of entrainment (Vollenweider et al., 2008). In these women, core body temperature and melatonin rhythms, validated markers of the internal clock, are phase-delayed compared with healthy controls (CON). The main physiological underpinning of PVD is the dysregulation of blood vessels. Under specific stimuli, such as cold, these individuals exhibit a contraction of acral vessels which results in cold extremities (Flammer et al., 2001). Given that vasculature in the skin is controlled by the autonomic nervous system (ANS), the question arises as to whether women with PVD and DIS (WVD) display a different pattern in the parasympathetic and sympathetic balance underlying cardiac control.

Within the framework of cardiac autonomic activity, heart rate variability (HRV) is a well known tool that provides a window onto autonomic modulation of the heart through frequency domain parameters that can be used to accurately interpret sympathovagal balance (Narita et al., 2007; Pagani et al., 1986; Pomeranz et al., 1985; Task Force of the European Society of Cardiology and the North America Society of Pacing Electrophysiology, 1996). Studies focusing on pharmacological interventions have

revealed that blocking the muscarinic parasympathetic transmission can abolish frequency peaks around 0.12-0.4 Hz and reduce the peak at 0.04 Hz in the power spectrum of heart rate (HR) fluctuations (Akselrod et al., 1981). Beta-sympathetic blockade, combined with raised arterial pressure, increased the peak at around 0.04 Hz (Akselrod et al., 1981). Taken together, this suggests that in HRV spectra the low frequency band (LF = 0.04-0.15 Hz) mirrors the influence of both sympathetic and parasympathetic activities, the high frequency band (HF = 0.15-0.4 Hz) is associated with parasympathetic activity, and the LF/HF ratio is indicative of sympathovagal balance (Task Force of the European Society of Cardiology and the North America Society of Pacing Electrophysiology, 1996).

However, there still is an open debate as to the functional significance of the very low frequency (VLF) band of HRV. The VLF band at 0.0033-0.04 Hz reflects even slower modulations of HR, and may represent the influence of the peripheral vasomotor and renin-angiotensin systems (Akselrod et al., 1981). It is also likely that VLF power is influenced by thermal stimuli (Fleisher et al., 1996). If this, indeed, is the case, one might expect for WVD to show differences to controls in this frequency domain.

Breathing plays a pivotal role on HRV output, particularly in the high frequency domain. Respiration shows a circadian modulation with troughs of respiratory variables at 6-8 h before the core body temperature minimum (Spengler et al., 2000). Furthermore, a higher power in the HF band occurs with increased respiratory rate, resulting in maximal power values for 12 breaths/min (Guzik et al., 2007). Only under fixed frequency breathing is it possible to make a clear distinction between LF and HF oscillations (Cooke et al., 1998).

There is evidence of the clustering of cardiac events between 06:00 to 12:00 h in the morning (after habitual nocturnal sleep) in a subset of arrhythmic patients (Goldstein et al., 1996; Gupta & Shetty, 2005; Marsh et al., 1990; Muller, 1999). Whereas the time course of HRV during a normal night's sleep is fairly well-known, it remains controversial as to whether the endogenous circadian rhythmicity of the sympathetic and parasympathetic nervous system exhibits a similar profile when people are sleep-deprived. Thus, we investigated the endogenous circadian rhythm of HRV parameters to examine how and in what direction the relationship between the sympathetic and vagal branch and/or the timing of the ANS between WVD and CON changes during sleep-deprivation under controlled constant-routine laboratory conditions.

Methods

Subjects

Eighteen healthy women (9 CON and 9 WVD) were selected to have had no shift work within 3 months or transatlantic flight within 1 month of the study, no medication (including hormones), or drug abuse, and all were non-smokers. Further exclusion criteria were extreme morning or evening chronotypes, amenorrhea or an irregular menstrual cycle. All volunteers were given questionnaires and a medical examination to determine physiological and psychological health. They completed a stringently controlled constant-routine (CR) protocol of 40 h prolonged wakefulness during the luteal phase. Chronotype was estimated using the Torsvall-Åkerstedt morning-evening-type questionnaire (Torsvall & Akerstedt, 1980).

The experimental protocol was approved by the local ethical committee (Human Research Committee of the Department of Medicine, University of Basel) and conformed with the ethical standards of the journal (Portaluppi et al., 2008). The main purpose and risks of the study were explained to the subjects before they gave their written consent. They could break off the experiment at any time. All subjects completed the study without any complaints.

Since there is strong body of evidence that acral skin temperature and skin temperature gradient are correlated with blood flow (House & Tipton, 2002; Rubinstein et al., 1990) or vasomotion (Severens et al., 2010), the screening for CON or WVD was not only conducted using subjective information about thermal discomfort with cold extremities (TDC), but also by finger temperature. Difference in vasomotion between CON and WVD was confirmed by the measurement of the distal–proximal skin temperature gradient before the baseline night. Additionally finger nailfold video capillary microscopy for objective assessments was carried out (criteria: blood standstill for ≥ 12 sec = women with PVD, < 12 sec = CON) (Emre et al., 2004; Gasser et al., 1991). DIS was evaluated by questionnaires, as well as via polysomnography during a screening night.

Design and Procedure

Within the screening procedure each woman spent a night in the sleep laboratory to confirm that they were able to sleep in the laboratory and to document whether the electroencephalogram (EEG) and electrocardiogram (ECG) patterns were normal.

One week before the experimental protocol began, subjects were requested to maintain a sleep-wake cycle according to their habitual bed time and to refrain from caffeine, alcohol, or extensive exercise (< 5 h/wk). Subjects came to the laboratory, 2 h before their habitual bedtime for an adaption night. They were prepared for sleep with ECG (V2, V5), EEG (C3,C4, Cz, Pz, Oz, Fz), EMG (chin), and EOG (outer canthi) electrodes, as well as a rectal probe (polyoxymethylene probe: 2-mm diameter, copper-constantan, Interstar, Cham, Switzerland; Therm, type 5500-3, Ahlborn, Holzkirchen, Germany) and eight skin temperature probes (silver disk: 1-cm diameter, copper-constantan, model P224, Prof. Schwamm, Ahlborn; Therm type 5500-3, Ahlborn, Holzkirchen, Germany). After awakening the subjects on the following day, the time until start of the protocol was used to adjust them to the experimental dim light conditions (< 8 lx). They were allowed to walk around the laboratory, but they had to wear sunglasses when walking out of the dimmed room to assure no strong light input.

Individual protocols started in the afternoon, 8 h before usual bedtime; the subsequent night was baseline night followed by an adapted (Mills et al., 1978) 40-h CR protocol and a recovery night. Participants remained in dim-light conditions (< 8 lx during wakefulness/0 lx during sleep) under constant semi-recumbent posture position in bed (head up not more than 45° during wakefulness/bed position 0° during sleep). Room temperature and relative humidity was kept constant at 2 °C and 55%, respectively. Subjects were studied singly, insulated from external sound or time cues; isocaloric sandwiches and water were administered at hourly intervals; non-overly stimulating neuropsychological tests were carried out every 2 h; and saliva and self-ratings of sleepiness (Karolinska Sleepiness Scale, KSS) were collected at half-hourly intervals. Blood pressure (BP) measurements were taken every hour (Ambulatory Blood Pressure Monitor, MOBIL-O-GRAPH® Vers.12, I.E.M GmbH, Stolberg, Germany).

In order to control breathing frequency, paced breathing (12 breaths/min = 0.2 Hz) was carried out within the CR protocol every 2 h for a duration of 3 min (20 paced breathing episodes/40 h). During the paced breathing episode, subjects were instructed to inhale and exhale naturally according to regularly 5 sec auditory signals (spoken voice recorded and rendered via a computer mediaplayer, inhale:exhale = 2:3 sec). Furthermore, each power spectrum was checked whether a peak in log power of HRV occurred at 0.2 Hz. During the breathing episodes subjects carried out the Karolinska Drowsiness Test, where movements are forbidden.

Data Acquisition and Data Analysis

Standard Ag-AgCl surface electrodes were placed on V2 and V5 (modified precordial lead). ECG was recorded with the VITAPORT ambulatory system (Vitaport-3 digital recorder, TEMEC Instruments B.V., Kerkrade, the Netherlands) with a sampling rate of 512 Hz. The raw signals were stored online on a Flash RAM card (Viking) and later downloaded off-line to a PC hard drive. A computerized system (System Hofstetter®, SHS Allschwil) was used to analyze the signals and detect the length of all R-R intervals over the entire 40-h CR, in particular for the 20 x 3 min paced breathing episodes. Each output was checked for plausibility by visual inspection. Obviously missing R-peak detections were replaced by interpolation of surrounding data and all R-R peak durations < 0.3 sec and > 1.8 sec were eliminated. If the noise was low, this time duration was taken for further HRV analyses. HR was calculated as 1/Median * 60 for all paced 3 min sequences.
Spectral analysis all 3-min sequences of R-R intervals were performed by a fast Fourier transform algorithm tapered with a Parzen window, after equidistant resampling with 16 Hz. Data were bidirectionally smoothed by a low pass filter with a cut-off frequency of 0.8 Hz. The power for VLF, LF and HF was calculated by the power spectrum. The LF/HF ratio was taken as a measure for the balance of the ANS.
Rhythm analyses were performed separately for both groups with ChronosFit (Zuther et al., 2009). This program combines a partial Fourier analysis and a stepwise regression technique. It fits the harmonics separately and carries out the F-test for each. Afterwards the most significant harmonic is included in the model. Additionally, nonorthogonal spectral analysis was used to fit the data to a two-harmonic model without correlated noise (Brown & Czeisler, 1992). Phases between 23-25 h were taken into account.

Statistics

Spectral power indices were logarithmically transformed to correct for a not-Gaussian distribution. The statistical packages Statview™ 5.0 (SAS® Institute Inc., Cary, USA) and Statistica™ 6 for Windows (StatSoft Inc., Tulsa, USA) were used. Time-course analyses were performed using two-way ANOVA for repeated-measures (rANOVA). The grouping factor was "group" (WVD vs. CON), and the independent variable was the factor time. Following rANOVA, all p-values were based on Huyhn-Feldt corrected degrees of freedom (reported are original degrees of freedom). Fisher's protected least significant differences procedure with alpha-correction for multiple comparisons (Curran-Everett, 2000) were calculated for post hoc comparisons. 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). The Mann-Whitney *U*-test was used to reveal significant differences between WVD and CON.

Linear regression analysis for comparing slopes of BP and HF power was performed. To examine the correlation of KSS values and log LF/HF ratio, data were pooled. A multiple linear regression analysis with repeated-measures was accomplished. The between-subjects differences were taken into account using dummy-coded subjects as forced variables in the model (Slinker and Glantz, 2008).

SigmaPlot® (Systat Software, Inc., Chicago, USA) was used for graphics, whereby values are presented as means ± standard errors (SE).

Results

Demographic Data and Physiological Characteristics

Groups were matched for age, body mass index, and chronotype ($p > 0.1$; Table 1). WVD had objectively measured significantly lower finger temperatures than CON (28.5 ± 0.99 °C vs. 32.83 ± 0.49 °C; $p < 0.05$) and prolonged sleep onset (self rated: 31.59 ± 4.46 min vs. 15.02 ± 3.27 min; PSG: 37.41 ± 10.47 min vs. 10.04 ± 1.14 min; $p < 0.05$). Distal-proximal skin temperature gradient was significantly decreased in WVD compared to CON (mean of 8 h before baseline night: – 3.08 ± 0.37 °C vs. – 1.87 ± 0.24 °C; $p < 0.05$).

Table 1: Subject's demographic data and habitual bed times

Variable	Age [yr]	BMI [kg/m^2]	Chronotype	Habitual light off times (clock time)	Habitual awakening times (clock time)
WVD	24.2 ± 1.2	20.82 ± 0.54	16.0 ± 1.6	23:25 ± 0:12	07:24 ± 0:12
CON	25.1 ± 1.7	20.85 ± 0.6	16.7 ± 0.9	23:46 ± 0:07	07:44 ± 0:07

Values are means ± SE. WVD = women with both, primary vascular dysregulation and difficulties initiating sleep. CON = controls. BMI = body mass index.

Heart Rate and Heart Rate Variability

In Figure 1 spectral analysis data are shown for all frequency bands. In WVD, paced breathing data revealed significantly lower log power values, especially in HF, but not in LF or VLF (except frequency band at 0.0056 Hz and 0.0444 Hz) with a breathing peak at 0.2 Hz.

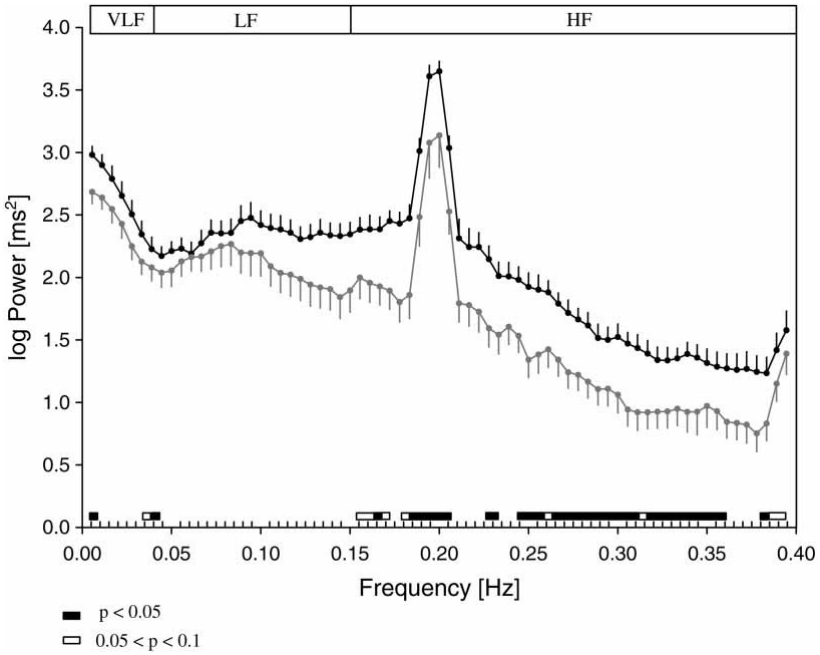

Figure 1 Power spectrum of heart rate variability. Data were averaged per constant routine episode for each subject before averaging across subjects (log mean ± SE, N = 9 each group; black line = CON; grey line = WVD). Horizontal black bars represent significant differences between WVD and CON ($p < 0.05$; open bars $p < 0.1$). The very low frequency (VLF) band includes frequencies between 0.0033-0.04 Hz, the low frequency (LF) band frequencies between 0.04-0.15 Hz and the high frequency (HF) band frequencies between 0.15-0.4 Hz.

The time-course of the HR and HRV parameters over the whole CR are shown in Figure 2. Significant differences between WVD and CON occurred in the HF band and the ratio of LF/HF. A tendency towards differences was found in the VLF band. The factor time was significantly different for HR, LF, HF, and LF/HF ratio. No significant interaction term between WVD/CON and time-of-day was found in HR or any HRV frequency band (Table 2). HR seems to be higher on the second day of the 40-h CR, but this could be ruled out by rANOVA comparing the first day of the CR (D1) with the second day of the CR (D2; $p = 0.13$). HR falls in the subjective night (sN) by about 6 bpm in CON and 3 bpm in WVD (peak of group values of D1 to trough of group values of sN).

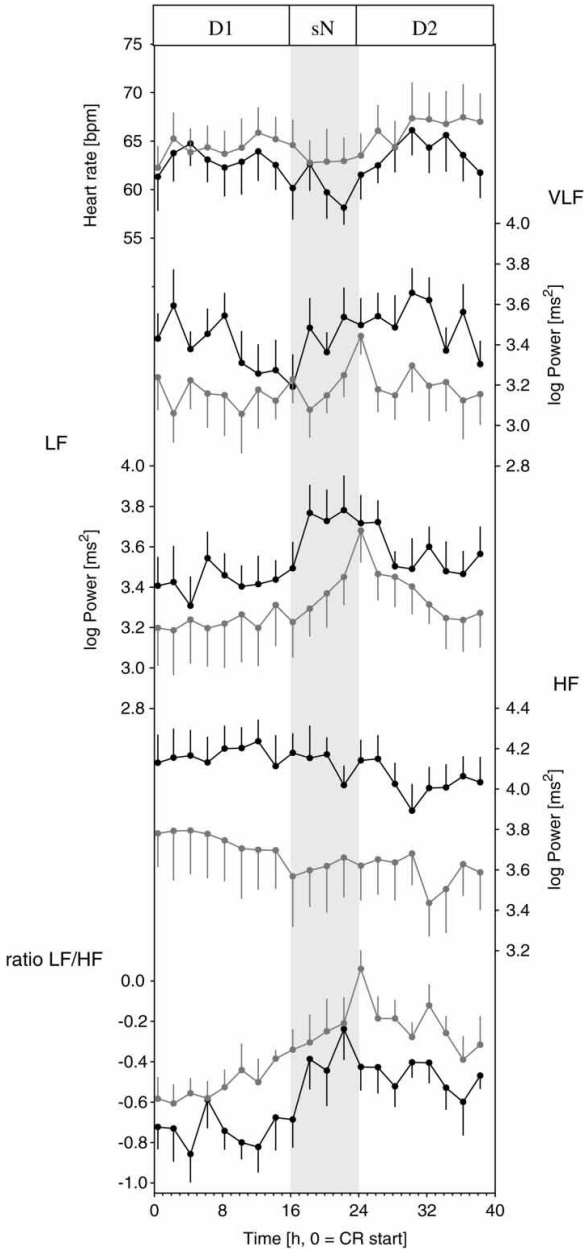

Figure 2 Time course (from top to bottom) of heart rate, log-transformed power of very low frequency (VLF, 0.0033-0.04 Hz), low frequency (LF, 0.04-0.15) and high frequency (HF, 0.15-0.4 Hz) –bands, and the LF/HF –ratio during the constant routine (CR) with 40 h of prolonged wakefulness. D1 = first day of CR, sN = subjective night (grey bar), D2 = second day of CR. (mean ± SE, black line = CON, grey line = WVD, N = 9 each group).

Table 2: ANOVA-table for HR and HRV variables

Variable	Group (df = 1, 16)		Time (df = 19, 304)		Time x Group (df = 19, 304)	
HR	F = 0.39	p > 0.1	F = 3.52	p < 0.05	F = 0.92	p > 0.1
VLF	F = 4.23	p = 0.056	F = 1.17	p > 0.1	F = 0.92	p > 0.1
LF	F = 1.67	p > 0.1	F = 3.22	p < 0.05	F = 0.72	p > 0.1
HF	F = 4.62	p < 0.05	F = 2.53	p < 0.05	F = 1.04	p > 0.1
LF/HF	F = 5.27	p < 0.05	F = 5.91	p < 0.05	F = 0.79	p > 0.1

Two-way rANOVAs with factors group (CON vs. WVD) and time (20 paced breathing episodes during CR) are shown for heart rate (HR), very low frequency power (VLF), low frequency power (LF), high frequency power (HF) and the sympathovagal balance (LF/HF).

Blood Pressure

The time-course of systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and the difference of SBP and DBP are plotted over the 40 h CR in Figure 3. The factor time was significantly different for DBP, but no significant difference in group or interaction term between WVD/CON and time-of-day was found in any BP variable (Table 3).

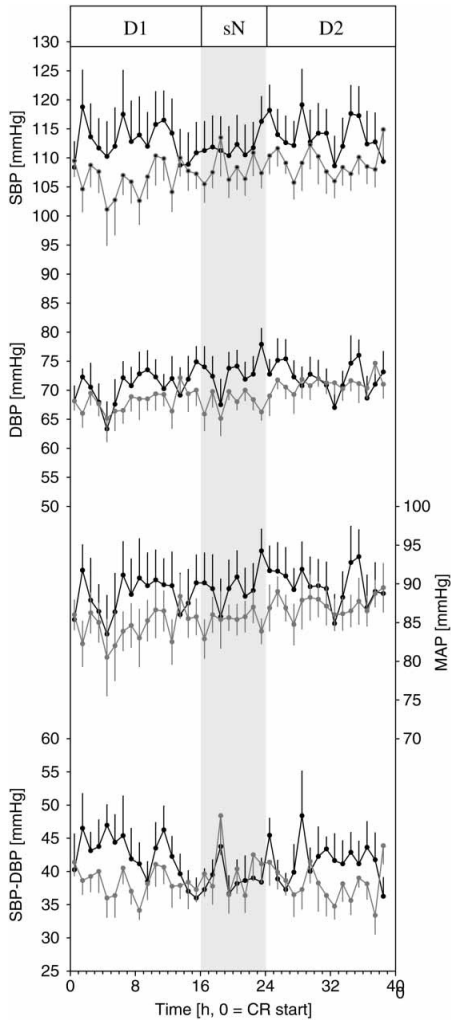

Figure 3 Time course of hourly measured blood pressure values during constant routine (CR) with 40 h of prolonged wakefulness. D1 = first day of CR, sN = subjective night (grey bar), D2 = second day of CR. From top to bottom: systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), pulse pressure (SBP-DBP; mean ± SE). Black line = CON; grey line = WVD (N = 8 each group).

Table 3: ANOVA –table for blood pressure variables

Variable	Group (df = 1, 14)		Time (df = 38, 532)		Time x Group (df = 38, 532)	
SBP	F = 0.89	p > 0.1	F = 1.16	p > 0.1	F = 1.12	p > 0.1
DBP	F = 0.71	p > 0.1	F = 1.68	p < 0.05	F = 1.15	p > 0.1
MAP	F = 0.80	p > 0.1	F = 1.38	p > 0.1	F = 0.98	p > 0.1
SBP-DBP	F = 0.80	p > 0.1	F = 1.25	p > 0.1	F = 1.36	p > 0.1

Two-way rANOVAs with factors group (CON vs. WVD) and time (39 time points during CR) for systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and pulse pressure (SBP-DBP).

Rhythm Analysis

A pooled rhythm analysis was performed due to the fact that no significant interaction term between group and time occurred in any parameter (Figure 4).

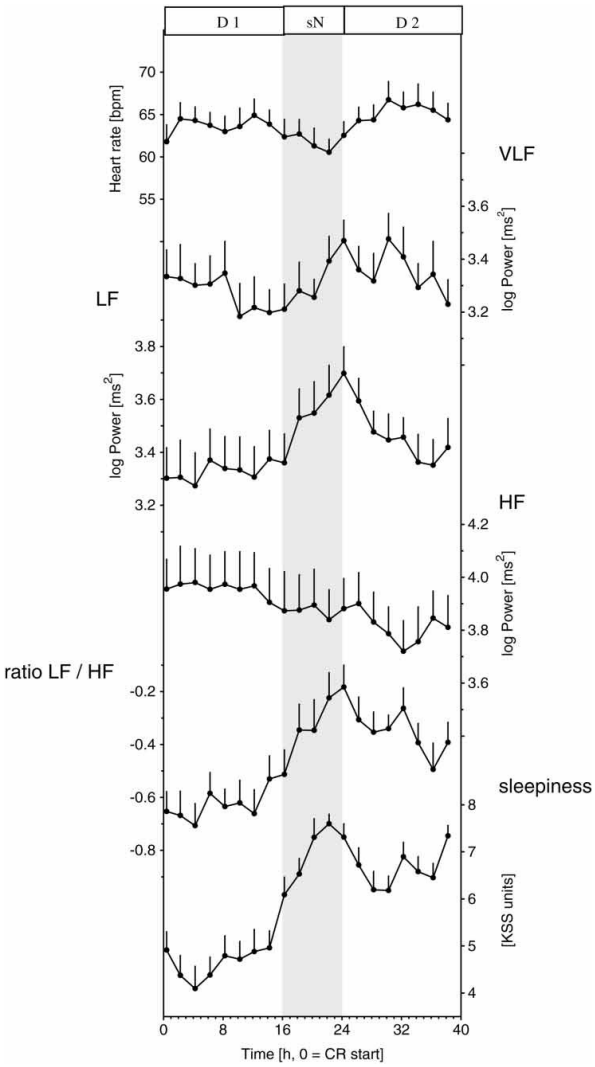

Figure 4 Time course of heart rate variables and sleepiness (pooled data, N = 18) during constant routine (CR) with 40 h of prolonged wakefulness. D1 = first day of CR, sN = subjective night (grey bar), D2 = second day of CR. From top to bottom: heart rate, log-transformed power of very low frequency (VLF), low frequency (LF), high frequency (HF), LF/HF -ratio and subjective ratings of sleepiness on the Karolinska sleepiness scale (KSS; mean ± SE).

ANOVA for the factor time revealed significance for HR, LF band, HF band, and LF/HF ratio. Within the paced breathing protocol, a circadian rhythm was observed in each of these parameters, except for the HF band (Table 4). The circadian rhythm of the mean HR shows a trough during the middle of sN, between 19.41 and 22.29 h after CR start. A broad maximum is seen in the afternoon. Maxima for circadian HRV parameters occur in the latter part of sN and minima are located in the late evening.

Not all subjects had a significant amplitude for the rhythm analysis and thus no circadian rhythm, but the mean of the fitted minima and maxima of individual CRs with significant amplitude after rhythm analyses are located in the similar time period. No significant circadian rhythm, but significance over time occurred in the HF band. The pooled data of

CON and WVD exhibit in the HF band a linear trend over time ($p < 0.05$, F = 12.55, df = 1,16). Despite the fact that log ratio of LF/HF exhibited a circadian rhythm, the same linear trend is evident ($p < 0.05$, F = 30.92, df = 1,16).

Table 4: Rhythm analyses

Parameter	Fitted Maximum of mean data with 95 % CI [h after CR start]	Fitted Minimum of mean data with 95 % CI [h after CR start]	Subjects with sign. amplitude	Mean Fitted Maxima of individual CR [h after CR start] ± SE [h]	Mean Fitted Minima of individual CR [h after CR start] ± SE [h]
HR	6.12 [-0.08 - 12.31]	20.85 [19.41-22.29]	14/18	10.25 ± 1.17	20.90 ± 0.50
LF	21.59 [19.42 – 23.77]	13.19 [5.86 – 20.52]	14/18	23.17 ± 0.94	12.32 ± 1.05
HF	n.s.		/	/	/
LF/HF	21.05 [16.73 – 25.40]	13.44 [4.45 – 22.43]	12/18	21.60 ± 0.74	12.29 ± 0.79

The table includes fitted maxima and minima of averaged data with 95 % confidence interval (CI; hours after constant routine start), subjects with a significant amplitude and averaged fitted maxima and minima of individual curves with standard error of the mean (SE) for heart rate (HR), low frequency power (LF), high frequency power (HF) and the sympathovagal balance (LF/HF). No significant (n.s.) amplitude for HF could be observed.

ANOVA for factor time revealed significance only for DBP. No nocturnal decline in BP was seen as found during sleep, but a linear trend with increasing slope for DBP was detected (F = 9.04; df = 1,14; $p < 0.05$).

Discussion

The present study is the first to examine differences in the ANS modulation of the heart between WVD and CON using HRV analysis. In order to control for interfering factors which might affect HRV, short-term (3-min) ECG recordings were performed during the Karolinska Drowsiness Test (Akerstedt & Gillberg, 1990) in a CR protocol of 40-h prolonged wakefulness. Using this protocol, environmental and physical conditions were kept constant and emotional as well as sensory stimuli were reduced to a minimum.

The key finding of this study is that HRV spectral analysis revealed specific differences between CON and WVD. During the course of 40-h wakefulness, WVD exhibited lower HF power and higher sympathovagal balance (LF/HF), with no differences in LF power, HR, and BP-parameters. In addition, significant circadian patterns were found selectively in HR

and LF power: HR, as previously found (Kräuchi & Wirz-Justice, 1994) was attenuated and LF power increased during the subjective night. Interestingly, these circadian variations did not differ between CON and WVD. BP (SBP, DBP and MAP) did not show any circadian variation. However, over 40-h of prolonged wakefulness a linear decrease occurred in the vagal branch of the ANS, and conversely DBP increased in a linear manner. The correlation between sleepiness and sympathovagal balance, which implies active regulation of the ANS against fatigue, is remarkable.

What are the theoretical underpinnings that can account for these specific group differences? WVD subjects were selected on the basis of thermal discomfort with cold extremities (TDC) and difficulties initiating sleep (DIS). Therefore, first it is discussed if TDC influences HRV and second the relationship between DIS and HRV. Finally, the circadian modulation of HRV and changes with increased elapsed time awake and supine position is considered.

How does TDC impact on HRV?

Several studies indicate that the thermal discomfort induced by cool environmental stimuli leads to changes in the HRV pattern (Liu et al., 2008; Nagashima et al., 2002; Yao et al., 2008). Vasoconstriction due to cool ambient temperature is mainly initiated from efferent skin sympathetic nerve activity resulting in a higher LF/HF ratio (Liu et al., 2008; Yao et al., 2008). Similarly, WVD in the present sample exhibit a higher LF/HF ratio compared to CON. Ambulatory and controlled laboratory studies have shown that WVD exhibit not only TDC, but also objectively measured lower distal skin temperatures (Gompper, 2007; Vollenweider et al., 2008). Thus, from a thermophysiological point of view, WVD live under a more vasoconstricted state in distal skin regions, which could be directly related to the higher sympathovagal balance.

Diminished perfusion of the distal vasculature in WVD leads to a proximal centralization of blood volume. Therefore heat content remains in the body core (proximal skin temperature and core body temperature are elevated in WVD compared to CON; recalculation of published data in Vollenweider et al., 2008). After bathing in warm water (39 °C), HF power decreases in CON (Kräuchi, unpublished data). Other studies have also shown attenuation of vagal activity when body heat content was elevated (Brenner et al., 1997; Bruce-Low et al., 2006). Thus, one might argue that changes in WVD thermoregulatory activity is associated with withdrawal of the vagal branch of the ANS.

An additional link between TDC and a different HRV pattern in WVD could evolve from a psychological point of view. Many emotional disorders (related to anxiety, fear, suppressed anger, panic etc.) have been associated with a reduction in HF power (Alvarenga et al., 2008; Bleil et al., 2008; Cohen et al., 2000; Friedman & Thayer, 1998a, 1998b; Johnsen et al., 2003; Klein et al., 1995; Marci et al., 2007; Ottaviani et al., 2009). A recently published study shows that a selective suppression of experienced anger was associated with TDC and increased sleep onset latency in women (Von Arb et al., 2009). Both, TDC and increased sleep onset latency were part of the inclusion criteria for WVD in the present study as well. Although anger suppression was not measured in the present sample, it might explain the relationship between psychological conditions and HRV. Therefore, prospective studies are needed to show whether anger suppression can explain the reduced HF power in WVD.

What is the role of DIS on HRV?

TDC in WVD seems to contribute to the decrease in the HF band, but does DIS also have an impact on HRV spectrum? DIS is one of the "Diagnostic and Statistical Manual of Mental Disorders" IV criteria for primary insomnia. Insomniacs with higher physiological arousal have – at least during sleep – an increased sympathetic activity and their vagal tone is decreased (Bonnet & Arand, 1998; Vgontzas et al., 1998). However, a recent CR study showed no significant differences in HRV during wakefulness between insomniacs and matched healthy sleepers (Varkevisser et al., 2005). WVD exhibit selective DIS, they do not, as insomniacs do, have problems maintaining sleep; nor do they manifest premature morning awakenings with a resultant reduced total sleep time and daytime sleepiness (Kräuchi et al., 2005). WVD do not show higher hypothalamic–pituitary–adrenal axis activity (same basal cortisol level; unpublished data) as often found in patients with insomnia (Buckley & Schatzberg, 2005). Therefore, a direct effect of DIS on HRV pattern seems rather unlikely.

From a chronobiological viewpoint, WVD exhibit differences in phase of entrainment. Their sleep-wake cycle does not differ from CON, but the circadian system is significantly phase-delayed (Vollenweider et al., 2008). A different phase angle leads to chronic circadian misalignment, which could lead to cardiovascular dysfunction – as found in shiftworkers (Boggild & Knutsson, 1999). A recent controlled laboratory study simulated the effect of circadian misalignment on the physiological pattern, but found no decrease in the HF band (Scheer et al., 2009). A short-term circadian misalignment may be quite different from chronic circadian misalignment as in our subjects.

What impact does time have on HRV – does a circadian rhythm exist?

Paced breathing HRV data revealed a linear decrease of vagal activity and a linear increase of sympathovagal balance with elapsed time awake and elapsed time lying in bed. Contradictory findings have been reported for these variables. In some studies with prolonged wakefulness, the output for the vagal branch of the ANS exhibited no decrease (Burgess et al., 1997; Holmes et al., 2002; Vandewalle et al., 2007), while other studies revealed an increase (Van Eekelen et al., 2004) or a decrease in vagal activity (Zhong et al., 2005). These different findings could be due to different protocols, such as different duration of time awake, controlled posture or not, or different parameters used for measuring activity of the vagal branch of the ANS. Our data follows that of the Zhong et al study (2005), since in protocols where sleep was allowed and subjects were not allowed to get up, no withdrawal of the vagal branch (Burgess et al., 1997; Kräuchi et al., 2000) or increase of sympathovagal balance could be established over time (Kräuchi et al., 2000). Thus, recumbency, *per se,* has no effect on vagal attenuation or of LF/HF ratio elevation. Taken together, our results support the hypothesis that vagal activity is responsive to homeostatic sleep pressure and additionally that sleep seems to be crucial for the regeneration of vagal activity and hence for a reduction of sympathovagal balance.

Our results are in accordance with other CR studies (Kerkhof et al., 1998; Van Dongen et al., 2001) with respect to the lack of day/night variations in BP during sustained wakefulness. Additionally, we also found a slight increase in DBP over time, which had never been previously reported. A higher DBP indicates a less than maximal dilatation of the arteries, which in turn could be in concert with the withdrawal of HF power over time (regression analysis: slopes of DBP vs. HF power, $r = -0.55$, $p < 0.05$; $N = 9$ pairs). BP regulation encompasses the interplay between the CNS, vasoactive molecules, baroreceptor sensitivity, HPA-axis activity, and the ANS. Every factor has its own rhythmicity and individual sensitivity (Portaluppi & Smolensky, 2001). Additionally, sleep has a profound influence on blood pressure. To what extent microsleep occurred in our subjects was not measured.

Breathing rates may provide the underlying reason for the non-existent circadian rhythm in HF power, which is in contrast to other studies (Burgess et al., 1997; Hilton et al., 2000; Vandewalle et al., 2007; Van Eekelen et al., 2004), particularly when one considers that these rates were the most apparent difference to other study protocols. If breathing is not paced, the rhythmicity of HF power might be due to the circadian rhythm in respiratory control. In fact, the circadian rhythm in respiratory control without sleep is known (Spengler

et al., 2000). If paced breathing is applied, respiration rate should be the same at all time points, which could abolish the circadian rhythmicity in HF power during prolonged wakefulness. Another distinction to other studies is sex of the subjects. To our knowledge, this is the first study in which all subjects were women in their luteal phase under CR conditions. It is known that women exhibit a different HRV pattern than men (Antelmi et al., 2004; Bonnemeier et al., 2003; John, 2007; Liao et al., 1995; Umetani et al., 1998), that the HRV pattern differs according to the hormonal cycle (Bai et al., 2009; Sato et al., 1995) and that women live under higher sleep pressure than men (Birchler-Pedross et al., 2009), which all might explain the linear decrease in HF power over time.

A circadian rhythm was found for LF power. To our knowledge only one study has previously analyzed the LF component of the HRV spectrum with respect to circadian variations in a constant-routine setting, and it also found a significant endogenous circadian rhythm (Vandewalle et al., 2007).

Due to the correlated curves for sleepiness and LF/HF ratio ($r = 0.53$, $p < 0.05$, $F = 7.29$; $N = 18$), one might argue that the ANS regulates against fatigue by an elevated sympathetic branch of the ANS during the night, when sleepiness is highest, seen in elevated LF power and a decrease in the vagal branch of the ANS during sN and on D2. This finding provides evidence that a CR protocol not only unmasks circadian patterns, such as core body temperature, but also induces masking effects on the ANS.

Limitations

Paced breathing occurred every 2 h over the CR protocol; therefore, HRV values were analyzed for the same timepoints. In contrast, BP was measured by the cuff method, using a standard sphygmomanometer every hour, not permitting analysis of the BP variability (BPV) for a direct sympathetic influence. In order to estimate the relationship between skin blood flow and activity of the vagal and the sympathetic branch of the ANS in a more conclusive way, different measurements have to be additionally considered in future studies, e.g., BPV, laser Doppler flowmetry, peripheral arterial tonometry. Respiratory volume was not measured; however, rhythms in the cardiovascular system were comparable with and without control of tidal volume (Cooke et al., 1998). One of the programs used for circadian phase estimation has been validated only for core body temperature or melatonin. To underpin the phases of HR and HRV indices, a 2nd program with a slightly different approach was used as well. Due to the relative small number of subjects, a larger sample is needed to confirm present data.

Conclusion

WVD manifest lower vagal activity and higher sympathovagal balance. The circadian rhythm of LF power showed a peak during the late night, which suggests that heart activity starts increasing prior to habitual awakening. This finding is seemingly related to the 24-h pattern of cardiac events, which occur most often immediately after awakening. However, reduced vagal and increased sympathovagal balance in WVD indicate an adverse physical constellation, which could be related to myocardial ischemia (Sroka, 2004) and to a higher predisposition for the development of coronary artery disease (Schwartz et al., 1992). Recent studies have shown that VLF is a predictor for myocardial ischemia as well (Bigger et al., 1992; Quintana et al., 1997). This parameter was slightly reduced in WVD. Taken together, WVD might be at a higher risk of developing heart failure due to an autonomic imbalance. Additionally the ANS in women seems to follow two strategies to fight against sleepiness. One is based on circadian control; the other based on homeostatic control, and both are reflected by the sympathovagal balance.

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Chapter 4

Relationship between heart rate variability, body temperature and electroencephalogram slow wave power during the sleep onset period in women.

D. Anders, B. Gompper, K. Kräuchi
Centre for Chronobiology, Psychiatric Hospital of the University of Basel, Switzerland

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Abstract

Cardiovascular and thermophysiological changes accompany the will to fall asleep. A relationship between core body temperature and heart rate variability especially during the sleep onset period is suggested, but only few data are available, investigating a relationship between skin temperature and HRV at this special time period. This study was aimed to elucidate the pattern of core body- and skin temperature, heart rate and its variability in healthy women having both, thermal discomfort from cold extremities and difficulties initiating sleep during the sleep initiation period during two subsequent nights in the laboratory. Furthermore, changes in sleep, temperature or cardiac regulation were examined after 16h of constant posture conditions. Data of 10 volunteers were investigated. Due to a faster decline of arousals the build-up of sleep stage 2, slow wave sleep and hence delta power is promoted in the second night compared to the first. Both, proximal and distal skin temperatures show an increase after lights out. Distal skin temperature at the time period around light out is already higher during the second night. Proximal skin temperature starts at the same temperature level for both nights but was significantly reduced in the second hour after lights out during night two. The distal-proximal skin temperature gradient, as a measure for distal dilatation of the skin vasculature, starts with a lower level after lights out in the first night, compared to the second. Different dynamics and differences between the two nights in skin temperature or sleep variables, but not in heart rate and HRV variables were found. Thus, a direct causality between all these functions seems rather unlikely in the present study sample. The described differences between both nights might occur from delayed relaxation, reflected in a slower decrease of arousals, prolonged sleep onset latency and a lower DPG at the first night. Especially the latter findings confirm nicely the statement that warm extremities promote a rapid onset of sleep.

Introduction

Several physiological human rhythms are influenced by the sleep-wake cycle. Studies with sleep episodes at daytime revealed, that skin temperature rises if naps are initiated [1], blood pressure decreases during a day-time nap [2] or heart rate decreases during daytime sleep [3]. Further, heart rate or core body temperature (CBT) decreases stronger during night time sleep compared to non-sleep studies [1]; [3]; [4]. Besides effects on body temperature, heart rate and blood pressure, changes of the cardiac autonomic activity

occur during sleep. In detail, the low frequency power of heart rate variability (LF, reflecting both, sympathetic and vagal influence on the heart) is high during arousals and REM sleep; the high frequency band (HF, reflecting the vagal influence on the heart) is high during NREM sleep [5-8]. Furthermore, delta waves of the electroencephalogram (EEG) were found to be inversely correlated with heart rate variability (HRV) variables, reflecting the balance of the sympathetic and vagal branch of the autonomic nervous system (e.g. LFnu or LF/HF ratio; [5]; [6]; [9]).

Large cardiovascular and thermophysiological processes occur during the period around lights out, when sleep is initiated. Physical (lying down) and mental (relaxing), as well as physiological changes accompany the will to fall asleep. The rapid decline of CBT in the late evening is suggested to increase the likelihood of sleep initiation [10]; [11]. Since CBT is the net result of heat production (well correlated with heart rate; [12]) and heat loss (due to foremost distal skin temperature vasodilatation; [13]). From a circadian point of view, the latter is predominant in the evening. In that it is shown, that skin temperature changes, as well as the decline of heart rate are initiated already prior sleep onset (reviewed in: [14]). Especially distal heat loss and thus an increase of the distal-proximal skin temperature gradient (DPG) prior sleep is a much better predictor for a short onset of sleep, than CBT [13], at least in young adults [15].

Only one study at present has determined the changes in temperature regulation and autonomic control of the heart occurring especially at sleep onset. LFnu and LF/HF ratio decreased prior sleep onset, while HF power increased. During sleep onset and sleep initiation no changes in HF power were observed, whereas LFnu and LF/HF ratio changed prior beginning of SWS and REM sleep, respectively [16]. CBT was significantly correlated with the LF/HF ratio prior sleep onset, but not after sleep onset or with the HF power. Thus, the authors concluded a relationship between CBT and HRV especially during the sleep onset period.

Women with a dysregulation in their distal vasculature respond to stimuli like cold or stress with vasoconstriction [17] and these subjects suffer more intensely from cold extremities [18]; [19]. An insufficient distal vasodilatation in the evening, often results in difficulties initiating sleep (DIS, [18-20]). Further, it is known that subjects who have primary vascular dysregulation and DIS have less parasympathetic activity, indicated by lower HF power of HRV analyses during a constant routine protocol with prolonged awakening [21]. However, it is presently unknown, whether HRV is affected during the sleep initiation period.

Although skin temperature, especially the gradient between distal and proximal skin temperature, is known to be a measurement for distal vasodilatation, controlled by the autonomic nervous system, the relationship between skin temperature and cardiac autonomic control during sleep initiation was not investigated yet. One caveat in studying such relationships derives from the population normally used for sleep studies. Good sleepers are often chosen, which are almost completely vasodilated before switching the lights off and fall asleep after few minutes. Therefore, the variance in these variables is low and bottom or ceiling effects after an intervention are highly probable. To overcome this, the first aim if the study was to elucidate the pattern of core body- and skin temperature, heart rate and its variability in women having both, thermal discomfort from cold extremities and DIS during the sleep initiation period during 2 subsequent nights in the laboratory. Furthermore, to our knowledge it was never questioned, whether changes in sleep, temperature or cardiac regulation occur after constant posture conditions without prolonged wakefulness. Therefore both nights were separated by controlled constant routine (CR) conditions in the laboratory, whereby subjects remained awake for 16h, according to their habitual sleep-wake cycle.

Methods

The following investigation was part of a larger study, involving 20 subjects with thermal discomfort from cold extremities associated with difficulties initiating sleep to examine the effect of two non-pharmacological interventions on sleep. Each intervention-part consisted of the same protocol, which encompassed a balanced design with baseline and intervention week. For analyzing differences between first and second laboratory night without any interventions, the present investigation focused on baseline nights before the intervention week started. Thus, the following data analyses are based on 10 participants, whose baseline week was prior the intervention week.

Subjects

Ten healthy young women (26.0±1.3 y; 20.5±0.4 kg/m^2; mean±SEM) were selected. None of them worked in shifts within 3 months or undertook transatlantic flights within 1 month prior to the study. Medication (excluding hormones) was prohibited. None of the participants had a history of drug abuse and smoking. Further exclusion criteria were extreme morning or evening chronotypes, amenorrhea or an irregular menstrual cycle. Chronotype was estimated using the Munich ChronoType Questionnaire (MCTQ). Five

women took contraceptive medication and five conducted the study in the luteal phase of the menstrual cycle. Additionally women were screened for thermal discomfort from cold extremities and difficulties initiating sleep by subjective information about temperature sensation and sleep behavior (screening criteria see [20]). All volunteers were given questionnaires to determine physiological and psychological health. The data revealed that all women were physically and psychologically healthy.

The experimental protocol was approved by the Human Research Committee of the Department of Medicine, University of Basel. The main purpose and risks of the study was explained to the subjects before they gave their written consent. Subjects could stop the experiment at any time. All subjects completed the study without any complaints.

Study design and procedure

Within 4 weeks before study begin, all subjects came for an adaptation night into the laboratory and completed an ambulatory screening week. Data based on actimetry data and sleep logs of the screening week were used to determine each subject's sleep times and confirm a habitual 8/16h sleep-wake cycle. Volunteers had to ensure a regular sleep-wake schedule, moderate physical activity and caffeine and alcohol abstinence during 4 days prior the study (compliance was checked via actimetry, sleep logs and questionnaires). Subjects came to the laboratory, 2h before their habitual bedtime. They were instrumented for sleep with ECG (V2, V5), EEG (C3, C4, Cz, Pz, Oz, Fz), EMG (chin), and EOG (outer canthi) electrodes, as well as a rectal probe (polyoxymethylene probe: 2-mm diameter, copper-constantan, Interstar, Cham, Switzerland; Therm, type 5500-3, Ahlborn, Holzkirchen, Germany). They laid down 30minutes prior lights out. Between the two 8h nights (referred to as N1 and N2, respectively) a 16h constant routine protocol was scheduled. Participants remained in dim-light conditions (< 8 lx during wakefulness / 0 lx during sleep) under constant semi-recumbent position in bed (head up not more than 45° during wakefulness / bed position 0° during sleep). Room temperature and relative humidity was kept constant at 22 °C and 55%, respectively. Subjects were studied singly and insulated from external sound or time cues. During the wake period isocaloric sandwiches were administered at hourly intervals; water ad libitum.

Measurements

Electrocardiography (ECG)

Standard Ag-AgCl surface electrodes were placed on V2 and V5 (modified precordial lead). ECG was recorded with the VITAPORT ambulatory system (Vitaport-3 digital

recorder, TEMEC Instruments B.V., Kerkrade, the Netherlands) with a sampling rate of 256 Hz. The raw signals were stored online on a Flash RAM card (Viking) and later downloaded off-line to a PC hard drive.

Polysomnography (PSG)

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) and downloaded offline to a personal computer hard drive.

Body temperatures

Core body temperature data were continuously recorded by a computerized system (Ahlborn) in 30-sec intervals. Skin temperatures from the following 13 sites were measured via wireless thermosondes (iButtons®): right and left side of the wrist at the radial artery above the os lunatum, left and right side above the calcaneus bone of the feet (together distal temperature), sternum, left and right infraclavicular, left and right groin (together proximal), left and right thigh and left and right calf. The distal-proximal skin temperature gradient (DPG; distal-proximal) was calculated as an index for vasodilatation of the skin vasculature.

Melatonin and Cortisol

Saliva collections (1-2 ml) were scheduled every 30 min, starting 4.5h before habitual bedtime at home (2 days prior the study). During the experiment saliva was collected every 30 min during wakefulness and twice per night (2h and 4h after lights out each). The samples were immediately refrigerated at 5°C, centrifuged within 2 days and stored at -20°C. A direct double-antibody radioimmunoassay was used for the melatonin analysis (validated by gas chromatography–mass spectroscopy with an analytical least detectable dose of 0.65 pm/ml; Bühlmann Laboratories, Schönenbuch, Switzerland). The cortisol concentration was measured by an enzyme-linked immunosorbent assay (Cortisol—Direct Salivary EIA; ALPCO Diagnostics, Salem, MA, USA) with a least detectable value of 1ng/ml.

Data analyses:

ECG

A computerized system (System Hofstetter®, SHS Allschwil) was used to analyze the ECG signals and detect the length of all R-R intervals over the 2 nights. Each output was checked for plausibility by visual inspection. Obviously missing R-peak detections were replaced by interpolation of surrounding data. If the noise was low, this time duration was taken for further HRV analyses.

For time domain, as well as frequency domain measures evaluating heart rate variability the Kubios HRV 2.0 software (Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland) was used.

In the time domain, the mean R–R interval, standard deviation of R–R intervals (STDRR) and root mean square of successive differences (RMSSD) were calculated for every 5-min R-R interval. HR was calculated as 1/R-R interval * 60.

Before spectral analysis of 5-min sequences of R-R intervals, a cubic spline interpolation was used. Data were re-sampled at 4 Hz to provide equidistant time points. The fast Fourier transformation spectrum in the software was calculated using a Welch's periodogram.

The values for the bands of the HRV frequency-domain analysis were defined as follows: VLF: 0–0.04 Hz, LF: 0.04–0.15 Hz and HF: 0.15–0.4 Hz. The LF/HF ratio, as well as LFnu (=normalized units: LF/(total power - VLF)) was taken as a measure for the balance of the ANS.

PSG

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. Sleep onset latency (SOL) was defined as the time interval between lights out and the occurrence of the first 20-sec epoch of sleep stage 2. REM sleep latency (REML) was calculated from sleep onset. Total sleep time (TST) was defined as stage 2 + 3 + 4 + REM sleep. Sleep efficiency (SE) was defined as follows: SE = TST/time between lights off and lights on x 100. Arousals were defined as: wakefulness + sleep stage 1 + movement time (MT) and expressed as percentage of the time between lights out and lights on. Sleep stages (2-4) and rapid eye movement sleep (REMS) were calculated as percentage of total sleep time (TST) during the respective night for all participants.

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. Especially the delta band (EEG power density in the range of 0.5-3.5 Hz) during non-REM sleep (NREMS) of the frontal midline derivation (Fz) was calculated.

Statistics

To correct for a not-Gaussian distribution, spectral power indices, sleep onset latency and REM latency were logarithmically transformed. Additionally the times spent in different sleep stages were transformed by taking the square root. Data were averaged to 5min bins. The statistical packages Statview® 5.0 (SAS® Institute Inc., Cary, USA) and Statistica® 6 for Windows (StatSoft Inc., Tulsa, USA) were used. Time-course analyses were performed using ANOVA for repeated-measures (rANOVA). The grouping factor was "night" (N1 vs. N2) and “time”. Huyhn-Feldt analyses was used to correct for violations of sphericity, all *p*-values were based on corrected degrees of freedom (reported are original degrees of freedom). The Duncan’s multiple range test was conducted for post hoc comparison. SigmaPlot® (Systat Software, Inc., Chicago, USA) was used for graphics, whereby values are presented as means ± standard errors (SEM).

Results

Every subject underwent at least 7h sleep during night 1 and night 2, respectively. Thus, 7h-mean values for sleep variables and values of body temperature and heart rate of the entire night are displayed in table 1. Melatonin concentration is shown as mean of both saliva collections each night.

Three subjects skipped their REM sleep either in N1 (two women) or in both nights (one woman) in the first sleep cycle. Thus, their values were set to duration of their NREM sleep in the first sleep cycle + 1epoch (20s). Two subjects had very long sleep onset latencies and therefore were woken up too early for saliva collection to reach REM sleep. These 2 participants were excluded from statistics for REML.

If values for both nights are compared, only sleep onset latency is statistically significant different, the gradient of distal and proximal skin temperature tend to show significant differences.

Variable	N1	N2	F(df)	*p*
SOL [min]	27.93 ± 5.93	13.13 ± 2.28	18.03 (1, 9)	0.002
REML [min]	96.42 ± 4.72	85.08 ± 8.72	2.12 (1, 7)	0.18
Arousals [%]	27.61 ± 4.74	17.97 ± 1.66	2.85 (1, 9)	0.13
S2 [%]	61.90 ± 1.83	60.97 ± 2.16	0.19 (1, 9)	0.67
S3 [%]	12.76 ± 1.07	13.09 ± 1.04	0.07 (1, 9)	0.80
S4 [%]	7.44 ± 2.21	7.96 ± 2.10	0.13 (1, 9)	0.73
SWS [%]	20.20 ± 2.34	21.04 ± 2.41	0.40 (1, 9)	0.54
REMS [%]	17.90 ± 2.10	17.99 ± 1.51	0.003 (1, 9)	0.96
SE [%]	72.39 ± 4.74	82.03 ± 1.66	2.85 (1, 9)	0.13
CBT [°C]	36.79 ± 0.08	36.81 ± 0.10	0.07 (1, 9)	0.80
DPG [°C]	-0.55 ± 0.07	-0.37 ± 0.09	3.27 (1, 9)	0.10
HR [bpm]	60.50 ± 2.08	59.45 ± 2.65	0.74 (1, 9)	0.41
MEL [pg/ml]	1.82 ± 0.17	1.52 ± 0.18	0.80 (1, 9)	0.40

Table 1: Sleep variables of 7h, temperature, heart rate and melatonin values per entire night are displayed. Data for CBT, DPG, HR and MEL were binned for every subject per night before repeated measure ANOVA was calculated. For SOL and REML original values are shown, but statistically analyzed with logarithmized values. Arousals are defined as wakefulness + sleep stage 1 + movement time and expressed as percentage of the time between lights out and lights on. The sum of sleep stages 2, 3, 4 and REM are amounted to 100%.
sleep onset latency (SOL); REM latency (REML); sleep stage 2-4 (S2-S4); slow wave sleep (SWS); REM sleep (REMS); sleep efficiency (SE); core body temperature (CBT); distal - proximal skin temperature gradient (DPG); heart rate (HR); melatonin (MEL)

Time course of sleep variables, body temperature and heart rate and its variability for the first 2hours after lights out, are displayed in Figure 1 A-C.

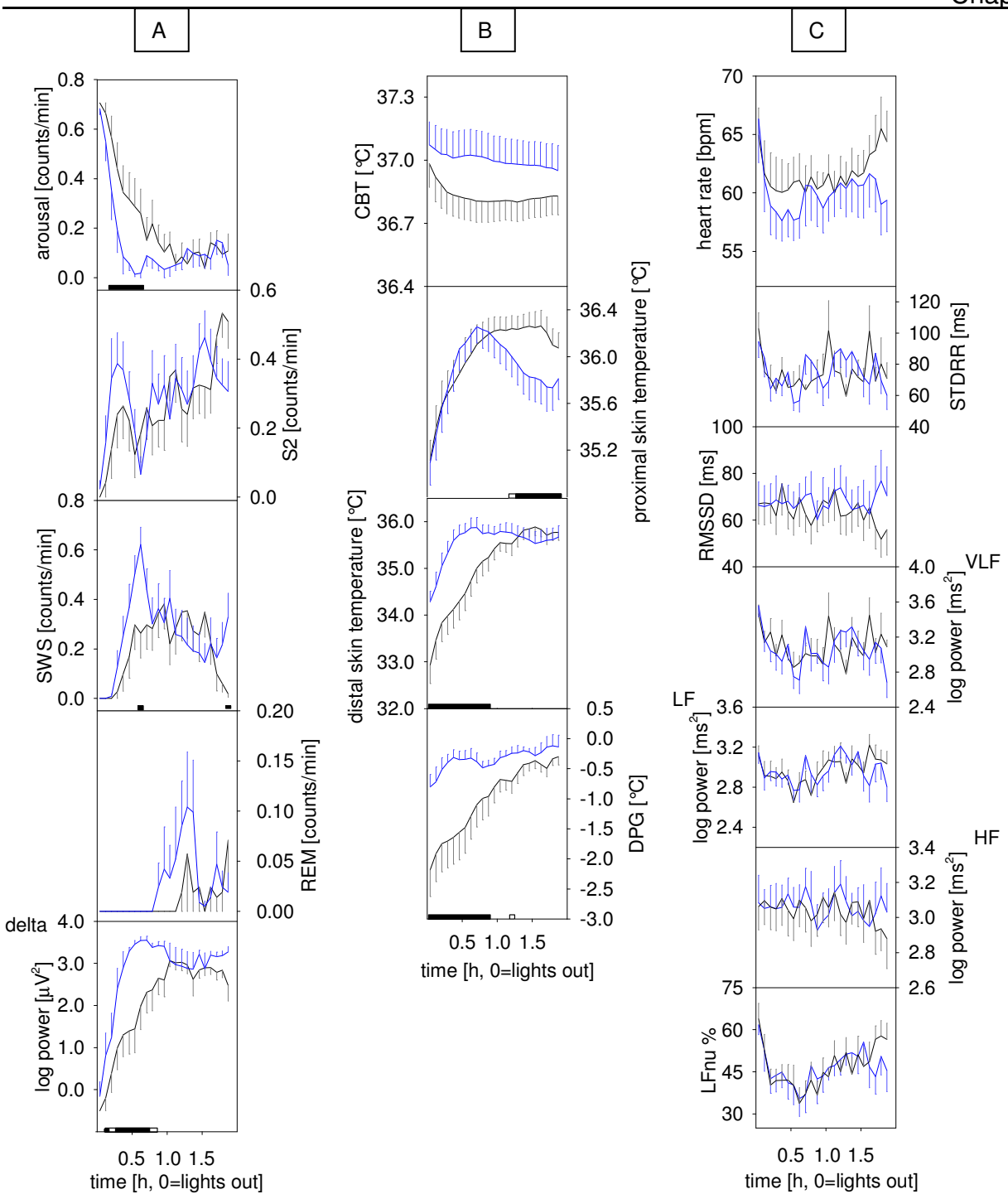

Figure 1A-C: Time course of physiological variables starting from lights out until 2hours after lights out. Black line = night 1; blue line = night 2. Horizontal black bars represent significant differences between night 1 and night 2 ($p < 0.05$; open bars $p < 0.1$).
1A: From top to bottom: Time course of arousal (awake+sleep stage1+movement time), sleep stage 2, slow wave sleep (SWS; sleep stage 3+4), REM sleep (REMS) and delta power density in NREM sleep for the frontal deviation of the central EEG (Fz)
1B: From top to bottom: core body temperature (CBT), proximal skin temperature, distal skin temperature, distal-proximal skin temperature gradient (DPG)
1C: From top to bottom: heart rate, standard deviation of R-R intervals (STDRR), root means square of successive differences (RMSSD), spectral power of very low frequency (VLF), low frequency (LF) and high frequency (HF), as well as the ratio of LF and HF

Due to a faster decline of arousals the build-up of S2, SWS and hence delta power is promoted in the second night compared to the first. Arousals revealed statistical

significance for the factor night (N1 vs. N2: F(1,9)=6.22, *p*=0.03) and night x time ($F_{arousals}$(22,198)=2.17, *p*=0.03). SWS tended to be different for night x time (F_{SWS}(22,198)=1.87, *p*=0.09). Post hoc comparison showed a significant difference from the 15th until the 40th minute after lights out for arousals and at the 40th and 115th min after lights out for SWS. For delta waves rANOVA revealed a significant main effect (night: F(1,9)=19.81, *p*=0.002), and a tendency for the interaction (night x time: F(22,198)=2.33, *p*=0.06). After post hoc comparison the times from 20min until 45min showed significant differences.

Although REM sleep seems to occur earlier and more often in N2 compared to N1 (Fig. 1A), it revealed neither a statistically significant main effect nor an interaction.

The core body temperature curve (Fig. 1B) declines faster during the first 2hours of the first night, but rANOVA revealed no significant interaction term between night and time (night: F(1,9)=2.28, *p*=0.17; night x time: F(22,198)=1.56, *p*=0.16).

Both, proximal and distal skin temperatures increase after lights out. Distal skin temperature at the time period around light out is higher during the second night. Proximal skin temperature at sleep onset was similar in both nights. After rANOVA a significant interaction term between night and time occurred for skin temperature (night x time: F_{distal}(22,198)=11.34, *p*<0.001; $F_{proximal}$(22,198)=4.49, *p*=0.001). Post hoc analysis showed a difference between N1 and N2 from minute 80 until 115 for proximal skin temperature while distal skin temperature show diverse developments immediately after lights out for 55 minutes.

The distal-proximal skin temperature gradient starts with a lower level after lights out in the first night, compared to the second. Factor night and night x time revealed statistical significance (night: F(1,9)=5.78, *p*=0.04; night x time: F(22,198)=4.44, *p*=0.02). Post hoc analysis revealed significant differences for the first 55min.

Neither heart rate, nor any HRV variable showed a statistically significant difference between N1 and N2, after rANOVA. Nevertheless, the curves for HR, LF power and LFnu revealed significance for the factor time (p<0.05). They start at a high level immediately after lights out and show a rapid decrease within 20 to 30 minutes (Fig. 1C).

Discussion

Cold extremities; hence distal vasoconstriction is displayed by the low distal-proximal gradient (DPG) at lights out of the first night. The vasoconstriction disappears and is not evident on the second night, confirming the data of diminished vasoconstriction during a

constant routine (CR) study with prolonged awakening in our laboratory [18]. The low DPG is mainly caused by sympathetic innervation of the distal vascular muscles. A changed balance in the activity of the autonomic nervous system was found by measures of heart rate variability (HRV, higher LF/HF ratio, [21]). Thus, an association between HRV and skin temperature could have been suggested. However, the present results indicate that a direct link between skin temperature or sleep variables and heart rate or HRV variables seems unlikely at the sleep initiation period. Not only the dynamics of the time courses are different (e.g. fast decrease in heart rate and LFnu immediately after lights out, but slow increase of skin temperature and slow wave sleep [SWS]), but also marked differences in sleep and skin temperature variables are present between the first and second night. Of note, autonomic control of the heart seems unaffected. Additionally, a relationship e.g. between core body temperature (CBT) and SWS is not evident.

In early work [22-24] it was hypothesized that a rapid fall or even an active down driving of CBT is linked to increased sleep stage 3 or 4. The present results do not support these observations. Although not significant, CBT showed a slower decrease in the second night compared to the first, but SWS was significantly higher 40min after lights out in the second night. An entire night comparison further revealed unchanged SWS. Thus, present data go in line with observations of Dijk and Czeisler, who found higher CBT in the recovery night, accompanied by more SWS [25].

However, some studies suggest that skin temperature is a better predictor for delta power density and slow wave sleep than CBT. In detail, passive warming of the skin, without elevating CBT was linked to an increase in SWS [26]. An imaging study of the human brain revealed, that after skin warming the hypothalamus is partly activated [27], an anatomical site associated with sleep-promoting neurons. Although the time course of skin temperature, especially proximal skin temperature in the present study, is similar to that of SWS and delta power density, no differences were found between first and second night.

Not only the relationship between sleep variables, body temperature and heart rate or HRV, but two different entrainment setting were elucidated. The first night includes the history of the entrained daily life (e.g. stress, light, large meals etc.), whereas the second night occurred after CR conditions, with constant dim light, supine posture and regular small food portions. Both nights have in common that they followed a 16h wake period, thus no difference in the homeostatic sleep pressure build-up should have been occurred.

Subjects remained in constant semi recumbent posture during the day in the laboratory and thus only minor posture changes occurred at the lights out period before the second

night. Contrary, volunteers changed their posture from upright position to lying position before the first night. A “lying down” effect has been described and was found to affect CBT for around 2h ([28]; [29]). Subjects in the present study laid down exactly 30minutes before lights out in the first night, thus the tendency of CBT to fall during the first 90min of the first night might be a result of the postural change. Heat redistribution from the core to the shell due to dilatation of the skin vasculature (seen e.g. by increasing DPG during the first night of the present data) has been suggested to promote these changes. Thereby heat distribution is linked to increased sleepiness, evaluated during times, when sleep pressure usually is very low (between 10am and 1pm; [29]). In the present examination data suggests that subjects did not profit from the lying down effect (slower decrease of arousals in the first night compared to the second). Furthermore, a reduced sympathetic outflow as described in literature [30] has not been identified by LFnu spectral power measurements in the present study. Thus, the lying down effect is unlikely to explain the observed data for HRV. This fact might be confirmed by another finding, derived using the same protocol apart from two additional laboratory nights one week prior. Data revealed no differences in arousals, SWS or delta activity between nights, but similar thermoregulatory effects (unpublished results).

An obvious further difference between the first and second night is given by its order. Studies investigating the “first night effect” in a sleep laboratory revealed a longer sleep onset latency (SOL, [31]; [32]). Although longer SOL in the first night compared to the second was analyzed in the present study, effects due to the “first night effect” can be ruled out. Subjects underwent an adaptation night prior the study and additionally neither difference in SWS nor sleep efficiency (SE) between the entire nights were found. Typically a decrease of SWS and less SE are found during sleep within the adaptation process to new environments [31]; [33]; [34]. Values of cortisol concentration revealed no statistical significance between nights (main effect “night”: $F(1,4)=1.32$; $p=0.32$), indicating that pronounced stress reaction effects in the first night, due to a new environment, can be excluded.

From a psychobiological point of view subjects of the present study were selected to have thermal discomfort with cold extremities that has been shown to be linked to turn the anger inwards [35]. This kind of stress management might be reflected in HRV during sleep [36], but is not in the present study. The decrease in LFnu as measure for the sympathetic cardiac modulation and HR immediately after lights out during both nights reflects, indeed, relaxation processes. On the other hand, the delayed relaxation after lights out in the first

night compared to the second is only seen in the slower decrease of arousals and in the lower temperature level of DPG, but not in LFnu or measures for vagal cardiac modulation (HF, RMSSD).

Another noteworthy result is that a lower DPG is accompanied by prolonged SOL, confirming previous findings that warm extremities promotes rapid onset of sleep [37].

Conclusion

The results of the present study revealed different dynamics of the time course and differences between the two nights in skin temperature and sleep variables, but not in heart rate and HRV variables. A direct causality between these variables seems rather unlikely in the present study sample. Studies, which investigated gender effects on HRV during sleep elucidated that women show a less pronounced increase of the LF/HF ratio during REM [38]; [39] compared to men. Thus, it might be concluded that foremost in women the central regulatory mechanisms of sleep, skin temperature and heart rate or HRV are rather weakly coupled.

However, night differences might not occur from the “lying down” effect, but from delayed relaxation, although this is only reflected in prolonged SOL, a slower decrease of arousals and a lower DPG during the first night, but has no effect on autonomic cardiac control.

Further investigations with different gender, as well as more than two consecutive nights in the laboratory could give more hints about the underlying mechanism between sleep, body temperature and cardiac regulation during the sleep onset period.

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Chapter 5

General discussion

The present thesis enclosed the aspect of the autonomic control of the human heart, influenced by thermoregulatory changes during constant routine protocols with and without sleep. The following discussion of the results will focus first on the physiological changes due to different thermoregulatory states and secondly circadian aspects will be considered. Last but not least gender aspects will be discussed.

In chapter 2 acute passive body heating (PBH) of young men resulted not only in an increase in core body temperature and a systemic vasodilatation but also in an increase in heart rate caused by the known effect of temperature elevation on the sinoatrial node (reviewed in: Cooper 1994). Additional analysis (see Annex) of comparison between intervention and the control group revealed that PBH induced a significant decrease in SDNN, a measure for global heart rate variability, suggesting that PBH led to a decrease of heart rate variability. The rMSSD, known as a measure for the vagal modulation to the heart, decreased. In line, the HF power of spectral HRV decreased; a measure that correlates with rMSSD. LF power was decreased, since it represents not only sympathetic but also vagal activity, LFnu was calculated. It has been suggested that this measure represents just the sympathetic branch of the ANS. The PBH-induced elevation in LFnu may indicate, that the sympathetic branch is activated, while the activity of the vagal branch is decreased.
These findings go in line with other observations, where PBH decreased the activity of the vagal part of the ANS (Brenner et al. 1997), leading to an increase of HR (Bruce-Low et al. 2006; Nagasawa et al. 2001; own unpublished data).
These data provide important basic physiological information for public health studies in extreme environments, health economy studies based on global climate change (Kjellstrom et al. 2009) or studies evaluating performance in athletes under warm ambient temperature.

It has been suggested, that specific thermoregulatory effect on VLF power is related to core hypothermia, rather than related to skin temperature changes (Fleisher et al. 1996). To present knowledge, few data on the change of VLF power at increase of core body temperature exist.

We have shown that VLF power decreases during PBH (chapter 2). In chapter 3, women with a dysregulation of the distal vasculature and a tendency to elevated CBT showed a tendency for a decrease of the VLF power during a CR protocol. These findings go in line with a study in hyperhidrosis patients, whose CBT were elevated. It was shown that in these patients the power at VLF is decreased (Birner et al. 2000). These facts might be a hint for VLF representing thermoregulatory changes. If so these thermoregulatory changes can have only minor impact on VLF power, due to several following observations. First, the difference of VLF power between groups during PBH sustained until 15.42-h after CR start compared to changes in CBT that sustained until 16.38-h after CR start. Second, during the first 2hours after lights off CBT declined. Therefore, reducing CBT in healthy subjects might result in an increase in power at very low frequencies, as suggested by Fleisher & co-workers (Fleisher et al. 1996). According to that, one might expect VLF power to increase. However, additional analyses (for chapter 4) indicate the opposite (data not shown). Therefore it can be concluded that additional factors are responsible for changes in the VLF power of the HRV analysis. In that, it has been suggested that VLF power might be a measure for the vagal part of the ANS (Taylor et al. 1998). Thus, the changes in VLF power presented earlier might additionally be due to short term oscillation of the ANS, since it goes in line with changes of HRV indices representing the vagal part of the ANS.
However, the interpretation of changes in VLF power can't be interpreted correctly as long as the mechanisms of this frequency range remains unclear.

If data of women with vascular dysregulation and difficulties initiating sleep (WVD) are compared with them of women without these symptoms, lower measures for vagal activity and higher sympathovagal balance were evaluated (chapter 3). No differences in LF power, reflecting combined sympathetic and vagal activation, heart rate, and blood pressure variables were found. However, as shown in other studies (Sroka 2004); (Schwartz et al. 1992), an autonomic imbalance indicates an adverse physical constellation that might be a predisposition for developing heart failure. Although the present thesis is based on a small number of subjects (N=9), one can't exclude that WVD might be a risk group for heart disease. Further examination is needed to determine risk factors.
Taken together, our results indicate that the primary vasospastic syndrome is based on much more complex dysfunctions of the autonomic nervous system than generalised sympathetic overactivity, as previously assumed (Vollenweider et al. 2008).

Studying women with thermal discomfort from cold extremities and difficulties initiating sleep during the sleep initiation period (chapter 4), differential dynamics in the time-course and differences between two consecutive nights in skin temperature or sleep variables, but not in heart rate and HRV variables were found. Although other studies suggest a relationship between thermoregulatory changes and HRV during the sleep initiation period (Okamoto-Mizuno et al. 2008), a direct causality between these functions seems rather unlikely in the present study sample. This might be due to the special subject group that was investigated or the utilisation of varying study protocols.
However, observed differences during the sleep initiation period of 2 consecutive nights in the study of chapter 4 might not occur from the “lying down” effect, but from delayed relaxation, although this is only reflected in a prolonged SOL, slower decrease of arousals and a lower DPG at the first night, but not in autonomic cardiac regulation. However, these data support once more the intimate relationship between distal vasodilatation and the rapid onset of sleep.

In the next section, the data are reconsidered from a chronobiological viewpoint.
Passive body heating (PBH) of young men in the late evening in chapter 2 revealed a phase advance in core body temperature, heart rate, and melatonin 24 hours after the intervention. The conduction of a CR protocol with prolonged awakening prevent the occurrence of main masking effects, like sleep, exercise or diet induced thermogenesis due to large meals.
Due to elevated blood flow triggered by warm ambient temperature, it can be assumed that PBH increases not only skin temperatures and CBT, but also brain temperature (Mellergard 1995, Whitten et al. 2009). Skin thermoreceptors (e.g. TRPV3) that detect ambient temperature and afferent C-fibres that travel to the brain (reviewed in Schepers et al. 2010), and also central thermoreceptors such as warm-sensitive ion channel receptors in the anterior hypothalamic brain region have been described. Additionally warm-sensitive neurons are found in the SCN (Van Someren 2003). Therefore, both peripheral and central thermoreceptors have the potential to transmit heat information to the SCN and provide the anatomical basis for a phase shift.
An efferent neural pathway from the SCN via the paraventricular nucleus, dorsal motor nucleus and intermediolateral column of the spinal cord to the heart has been described (Scheer et al. 2003). This pathway might be responsible for the steeper and earlier decline of the HR on the second day, seen as a tendency in the intervention group (chapter 2).

Interestingly, HRV measures were similar between groups, suggesting that a possible shift of the SCN, induced by the PBH procedure did not influence the autonomic cardiac control. Heart rate is an outcome measure of the activity of the sinoatrial node, its firing rate is temperature dependent. Thus, the phase shift of HR might have occurred passive due to elevated CBT and hence a faster firing rate of the pacemaker cells.

In chapter 3, significant circadian patterns were found exclusively for HR and LF power. In line with previous results, HR was attenuated (Krauchi et al. 1994) during the subjective night. The circadian rhythm of LF power showed a peak during the late night, which suggests that heart activity increases prior to habitual awakening. This finding is seemingly related to the 24-h pattern of cardiac events, which occur most often immediately after awakening. BP (SBP, DBP, and MAP) did not show any circadian variation. The last finding is in accordance with Kerkhof and collaborators and Van Dongen and collaborators (Kerkhof et al. 1998; Van Dongen et al. 2001). In contrast, a more recent study showed circadian variation in BP (Scheer et al. 2010). Of note, the latter study was conducted using a forced desynchrony protocol, while a constant routine protocol with prolonged awakening was utilized in the earlier investigations. Therefore the examination protocol might have an impact on the measurement of rhythmicity of BP.

The circadian rhythmicity, found for LF power in chapter 3 is in line with Vandewalle and collaborators (Vandewalle et al. 2007). Since LF power reflects both, vagal and sympathetic activity, but the first has no circadian rhythm (rather linear slope of HF power – chapter 3) one might assume a prevailing circadian effect on the sympathetic part of the ANS. A possible underlying mechanism might be a circadian rhythm of noradrenaline plasma levels (e.g. Scheer et al. 2010), with low levels during the evening / beginning of the subjective night and increasing levels during the end of the subjective night, hence resulting in a circadian rhythm of the sympathetic branch.

Taken together, a direct influence from the SCN to the autonomic cardiac control seems rather unlikely. This assumption is supported by a study showing abolished rhythmicity of HRV measures in the absence of a functioning SCN, which was not restored after transplantation of an intact SCN, in contrast to rhythmicity of temperature measures that were restored (reviewed in Dibner et al. 2010).

The forth chapter has shown profound differences in sleep and temperature variables, but not in HRV measures between two consecutive nights in the chronobiological laboratory. One difference between the nights was previous light history. The time spent awake before

the first night has been undertaken under daily life light exposure, while the time awake before the second night was under dim light conditions. Importantly melatonin concentrations were similar in both nights. Thus, prior light history seems to have no effect on melatonin secretion. The timing of the inner clock, represented by the dim-light melatonin onset, measured at home compared to the corresponding time in the laboratory, has revealed a delayed increase of melatonin concentration at home (clock time: 23:09 vs. 22:35). Light perception during daily life leads typically to entrainment of the whole orchestra between e.g. sleep onset and bodily functions (decline of CBT, HR and sympathetic measures, rise of skin temperature and vagal activity). The longer sleep onset latencies observed in the first night, displaying the effects of an ambulatory situation. It might be speculated that subjects were not entrained well during daily life. However, times for dim light melatonin onset were not statistically significant different (repeated measures ANOVA, main effect “night”: $F(1,9)=1.88$, $p=0.2$). Therefore it remains speculative whether the status of entrainment has indicative potential in this context.

Although none of the previous studies included mixed gender groups and therefore no direct comparison of gender is possible; the last section encompasses some general gender aspects on HRV analysis.

It is commonly agreed that sex and ovarian cycle affects the HRV pattern (Antelmi et al. 2004, Bonnemeier et al. 2003, John 2007, Liao et al. 1995, Umetani et al. 1998, Bai et al. 2009, Sato et al. 1995). These differences might help to explain some findings in chapter 3 (i.e. the correlation between sleepiness and sympathovagal balance, which implies active regulation of the ANS against fatigue).

Studies, investigating gender effects on HRV during sleep elucidated that women show a less pronounced increase of the LF/HF ratio during REM (Valladares et al. 2008; Elsenbruch et al. 1999) compared to men. Thus, it might be concluded that in women the central regulation of sleep, skin temperature and heart rate or HRV demonstrate a rather weak mechanistic coupling (chapter 4).

Conclusion

The present thesis provides insight into the relationship between cardiac control and thermophysiology due to different methods (constant routine protocols with prolonged awakening or two subsequent sleep episodes divided by 16h awakening), by an

intervention (passive body heating) or by the special screening for subjects (inherent a 'model of nature' representing thermoregulatory related alterations).

Passive body heating revealed an acute effect on body temperature and HRV measures. In detail the variability of the heart and vagal activity were attenuated. On the other hand measures known to reflect sympathetic activity and core body temperature were increased; and systemic vasodilatation occurred. Results of women with primary vascular dysregulation revealed, that HRV measures during demanding situations (e.g. prolonged awakening) showed adverse physical constellations. Further an uncoupling between thermoregulation and HRV measures during the sleep onset period was seen. The thesis provides evidence that HRV measures were hardly influenced by the SCN, but by thermoregulation.

Furthermore, the present thesis adds new aspects to the ongoing debate about the VLF range of HRV. In this it seems very likely that VLF measures reflected not thermoregulatory processes alone, but also cardiovascular regulations.

To set the scene into a global context it is important to know the interaction between thermoregulation and cardiovascular regulation. One example might be the pharmaceutical field. Therapeutic drugs are available that influence CBT (Buhr et al. 2010). But not much is known about the resulting cardiac impact of these drugs that occur if thermoregulation is influenced. Therefore the present thesis enlarges the basic knowledge about the interaction between autonomic cardiac control and thermoregulation and hence might establish a basis for e.g. upon constructive studies.

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Annex

Contribution - chapter 2

*These authors contributed equally to this work (contribution DA: planning, realisation, analysis of the pilot-study; planning, realisation of the study for 4/8 subjects; analysis of all HRV data; ANOVA, as well as the Huynh-Feldt (H-F) statistic for all data; analysis of melatonin data; publication management; writing the manuscript together with BG and KK).

Supplemental methods – chapter 2

For heart rate variability time domain, as well as frequency domain measures the Kubios HRV 2.0 software (Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland) was used.

Within the time domain the standard deviation of R–R intervals (SDNN) and root mean square of successive differences (rMSSD) were calculated for every 5-min interval.

Before spectral analysis of 5-min sequences of R-R intervals, a cubic spline interpolation was used. Data were re-sampled at 4 Hz to provide equidistant time points. The Fast Fourier Transformation spectrum in the software was calculated using a Welch's periodogram.

The frequencies of the HRV frequency-domain analysis were defined as follows:

Very low frequency (VLF: 0–0.04 Hz), low frequency (LF: 0.04–0.15 Hz) and high frequency (HF: 0.15–0.4 Hz). The LFnu (=normalized units: LF/(total power - VLF)) was taken as a measure for the sympathovagal balance.

Supplemental results – chapter 2

Baseline

The same analysis, as for CBT, HR and DLMO were conducted for SDNN and rMSSD. Two-way ANOVAs for repeated measures for data of the 11-h time segment starting 2.5-h after lights on revealed the following results:

SDNN GROUP: $F_{(1,14)} = 0.14$, $p = 0.71$

rMSSD GROUP: $F_{(1,14)} = 0.17$, $p = 0.68$

Acute effect

Supplemental table 1

Variable	Group		Time		Time x Group	
SDNN	$F_{1,14} = 0.67$	p = 0.43	$F_{8,112} = 13.01$	p < 0.001	$F_{8,112} = 13.06$	p < 0.001
rMSSD	$F_{1,14} = 6.03$	p = 0.03	$F_{8,112} = 11.97$	p < 0.001	$F_{8,112} = 9.03$	p < 0.001

ANOVA for repeated measures were calculated for SDNN and rMSSD for the ACUTE-effect, meaning the time segment between 13.5-h after lights on and 18-h after lights on (=time segment including data before intervention [1-h], during [0.5-h] and acute after-effects [3-h]).

Post-treatment day

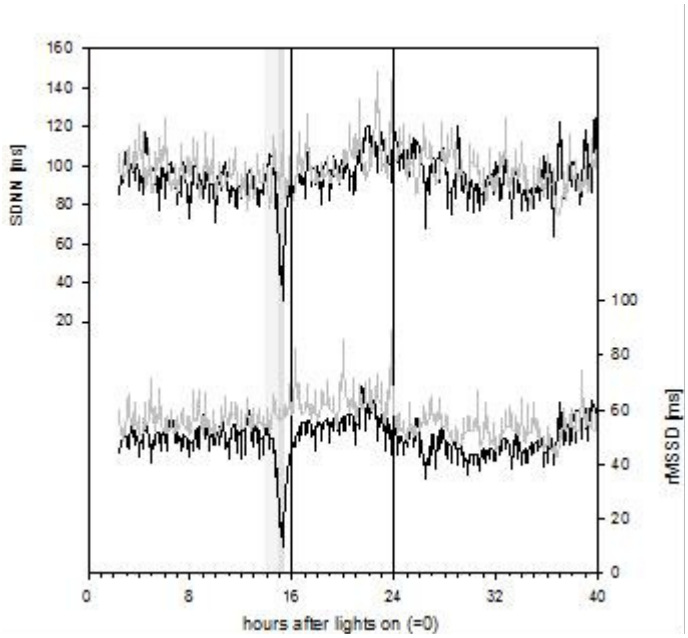
Two-way ANOVAs for repeated measures for data of the 11-h time segment starting 26.5-h after lights on revealed the following results:

SDNN GROUP: $F_{(1,14)} = 0.14$, p = 0.71

rMSSD GROUP: $F_{(1,14)} = 0.17$, p = 0.68

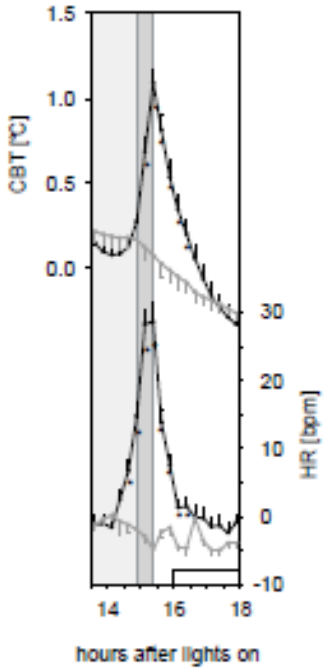
Supplemental figures – chapter 2

Supplemental Figure 1



Supplemental Fig. 1 Time course (mean ± SE) of standard deviation of normal RR intervals (SDNN) and root of mean squared successive differences (rMSSD; 15-min bins) and salivary melatonin values (in half-hourly intervals) for the intervention group (black; N = 8) and controls (grey; N = 8) during a constant routine protocol with 40-h sustained wakefulness (data are plotted from 2.5 h to 40 h after lights on = 0). The intervention (warm bath, 39°C) is indicated by the grey bar, subjective night (where they would have slept) by the blank area between two vertical lines, and the time of masking effects due to preparing for the intervention by the light grey bar.

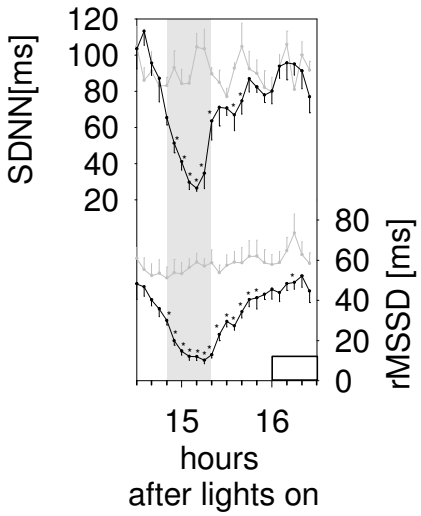
Supplemental Figure 2

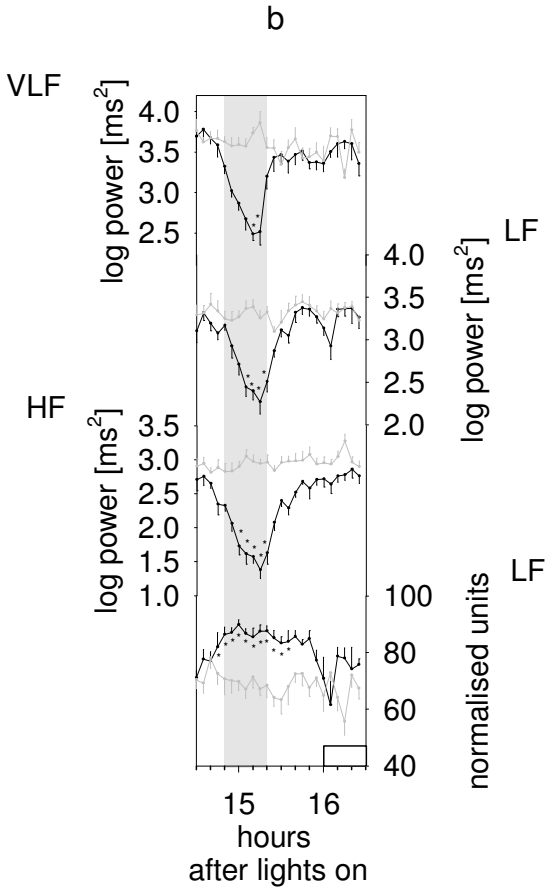


Supplemental Fig. 2 Acute effects of passive body heating, displayed by core body temperature (CBT) and heart rate (HR; mean ± SE; 15min bins). Asterisks indicate significant differences between intervention group (black) and controls (grey). Intervention is indicated by the grey bar, first part of the subjective night by the blank vertical bar and time of masking effects due to preparing for the intervention by the light grey bar.

Supplemental Figure 3

a





Supplemental Fig. 3 Acute effects of passive body heating, displayed by a) standard deviation of normal RR intervals (SDNN) and root of mean squared successive differences (rMSSD), b) very low frequency (VLF), low frequency (LF), high frequency (HF) and LF normalised units (mean ± SE). Asterisks indicate significant differences between intervention group (black) and controls (grey). Intervention is indicated by the grey bar, beginning of the subjective night by the blank vertical bar.

Curriculum Vitae

Personal Details

Name:	Doreen Anders
Date and Place of birth:	August 28, 1979 in Suhl, Germany
Residence:	Allschwil, Switzerland
Citizenship:	German

Education

10/2006 – 10/2010	PhD student at the Centre for Chronobiology, Psychiatric Hospital of the University of Basel, Switzerland under the supervision of Kurt Kräuchi
11/2005	Degree and license as pharmacist
10/1999 – 10/2004	Studies in pharmacy, University of Jena, Germany

Work Experience

02/2011 – present	Employment as project manager, archimed medical communication ag, Zofingen, Switzerland
06/2006 – 10/2010	Employment as research assistant at the Thermophysiological Chronobiology Centre for Chronobiology, Psychiatric Hospital of the University of Basel, Switzerland
03/2006 – 05/2006	Employment as pharmacist, Avie Apotheke, Singen, Germany
05/2005 – 10/2005	Practical pharmaceutical training in a community pharmacy, Wasserturm Apotheke, Mannheim, Germany
11/2004 – 04/2005	Practical pharmaceutical training in a pharmaceutical company, Abbott GmbH & Co.KG, Ludwigshafen, Germany Research project: Properties of proteins during cooling and heating using DSC (differential scanning calorimetry)
09/2003	Industrial placement at THC-Pharm GmbH, Frankfurt/Main, Germany Research assistant (working area: research and development)
07/2003	Internship in the dispensary SRH Zentralklinikum GmbH, Suhl, Germany
05/2003	Research assistant at the Department of Biological Pharmacy, University of Jena, Germany

Grants and Certifications

09/2010	Travel Grant of the Swiss Society of Sleep Research, Sleep Medicine and Chronobiology

28.08.2009	Poster prize of the European Biological Rhythms Society in Strasbourg, France
08/2009	Registration fellowship of the Société Francophone de Chronobiologie
09/2008	Travel Grant of the Swiss Society of Sleep Research, Sleep Medicine and Chronobiology
29.09.2007-06.10.2007	Certification for the participation in the 2nd Euclock and 16th European PhD School for Chronobiology
31.07.2006-08.08.2006	Certification for the participation in the VIIth International Course on Chronopharmacology

Memberships in Professional Societies

since 2009	Member of the Societé Francophone de Chronobiologie
since 2005	Member of the German Pharmaceutical Society (DPhG)

Publications and Presentations

Papers in peer-reviewed journals

Christian Cajochen, Sylvia Frey, **Doreen Anders**, Jakub Späti, Matthias Bues, Achim Pross, Ralph Mager, Anna Wirz-Justice, Oliver Stefani
Evening exposure to a light emitting diodes (LED)-backlit computer screen affects circadian physiology and cognitive performance.
J Appl Physiol, published online before print March 2011, http://jap.physiology.org/cgi/content/abstract/japplphysiol.00165.2011v1

Anders D, Gompper B, Kräuchi K
Relationship between heart rate variability, body temperature and electroencephalogram slow wave power during the sleep onset period in women.
Physiology & Behavior, submitted

Anders D, Gompper B, Knoblauch V, Wirz-Justice A, Cajochen C, Kräuchi K
Is an evening heat pulse a zeitgeber in humans?
PLoS ONE, submitted

Anders D; Vollenweider S; Cann J; Hofstetter M; Flammer J; Orgül S; Kräuchi K
Heart rate variability in women during 40 hour prolonged wakefulness.
Chronobiology International 2010, 27:8, pp1609-1628

Abstracts in peer-reviewed journals

Anders D, Gompper B, Kräuchi K
Aligning the internal phase of entrainment shortens sleep onset latency and deepens sleep, accompanied with less hyperarousals. 20th Congress of the European Sleep and Research Society (ESRS), Lisbon, 14.09 -18.09.2010. J.Sleep Research , 19 (Suppl. 2), 1–378

K. Kräuchi, **D. Anders**, B. Gompper and S. Vollenweider
Phase of entrainment and sleep initiation from a thermoregulatory point of view. 20th Congress of the European Sleep and Research Society (ESRS), Lisbon, 14.09 - 18.09.2010. J.Sleep Research , 19 (Suppl. 2), 1–378

D Anders, S Vollenweider, A Wirz-Justice, J Flammer, S Orgül and K Kräuchi
Heart rate variability analyses of women with vasospastic syndrome and difficulties initiating sleep exhibit lower vagal nerve activity. 19th Congress of the European Sleep and Research Society (ESRS), Glasgow, 09.09 -13.09.2008. J.Sleep Research , Vol. 17, Abstr. Supplement 1, Dec.2008

Anders D, Vollenweider S, Hofstetter M, Wirz-Justice A, Orgül S, Flammer J, Kräuchi K
Women with difficulties initiating sleep and vasospastic syndrome exhibit lower heart rate variability in the high frequency band. 22 nd Annual Meeting of the Associated Professional Sleep Societies (APSS), Baltimore, 07.06-12.06.2008.Sleep. 2008; (Abstr. Suppl.) 31: A29

Anders D, Vollenweider S, de Quervain DJ, Wirz-Justice A & Kräuchi K
Effects of 40-hour sleep deprivation on short-term memory in a constant routine protocol. 21st Annual Meeting of the Associated Professional Sleep Societies (APSS), Minneapolis, 08.06. - 14.06.2007. Sleep. 2007; (Abstr. Suppl.) 30: A373

Oral presentations

Anders D: Heart rate variability analyses of women with vasospastic syndrome and difficulties initiating sleep exhibit lower vagal nerve activity. 19th Congress of the European Sleep and Research Society (ESRS), Glasgow, 13.09.2008

Anders D: CR Berlin – Circadian rhythms and masking effects (Melatonin and skin temperature), 4th ClockWork Meeting Ladenburg, 07.12.2007

Anders D: Preliminary analysis of CR investigations of the digit span test, 3rd ClockWork Meeting Ladenburg, 14.11.2006

Poster presentations

BioValley Life Sciences Week, Basel, 22.09.2010
20th Congress of the European Sleep and Research Society, Lisbon, 17.09.2010
Clock Work Final Meeting, Berlin, 16.06.2010
Swiss Society of Sleep Research, Sleep Medicine and Chronobiology, Lausanne, 13.03.2010
XIth Congress of the European Biological Rhythms Society, Strasbourg, 23.08.2009
5th Clock Work Meeting, Ladenburg, 25.11.2008
International Society for Psychoneuroendocrinology, Dresden, 18.07.2008
Swiss Society of Sleep Research, Sleep Mecicine and Chronobiology, Solothurn, 06.03.2008
Swiss Society of Biological Psychiatry, Basel, 15.03.2007
Swiss Society of Sleep Research, Sleep Medicine and Chronobiology, Lausanne, 22.02.2007