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Sleep-wake and circadian-dependent variation of cardiorespiratory coherence

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Abstract—**The risk of adverse cardiovascular events is elevated in the morning compared to the rest of the day. A circadian and a sleep-wake dependent variation in the regulation of the cardiovascular system could contribute to this increased cardiovascular risk. Using an ultradian sleep-wake cycle (USW) procedure, our aim was to explore the effects of the sleep-wake and circadian cycles on cardiorespiratory coherence (CRC) as a measure of autonomic nervous system (ANS) state. Our results suggest a shift toward parasympathetic dominance with deepening of sleep. Conversely, REM sleep is associated with a sympathetic dominance comparable to levels observed during wakefulness. A circadian rhythm was observed for CRC during wakefulness and all sleep stages. Maximal sympathetic dominance was observed in the morning, as measured by CRC during wakefulness and REM sleep, consistent with studies showing increased cardiac risk in the morning. This study provides evidence that circadian and sleep processes interact to influence the ANS modulation of the heart.**

I. Introduction

There is a clear diurnal (day/night) pattern of adverse cardiovascular events, with higher risk between 06:00 and noon as compared to the rest of the day [1]. Even though this peak in cardiovascular vulnerability corresponds to the habitual time of awakening, the onset of daily activity cannot entirely account for the 24-h distribution of myocardial ischemia [2], suggesting a role for the circadian pacemaker.

A wide range of physiological processes demonstrate circadian rhythms (endogenous and entrainable rhythms of ~ 24 h). These rhythms are driven by an endogenous central pacemaker located in the suprachiasmatic nucleus of the hypothalamus. The circadian system orchestrates the diurnal variation of a variety of biological and physiological systems, including core body temperature (CBT), cortisol secretion, sleep propensity, cardiovascular parameters. Heart rate (HR) has been shown to follow a circadian rhythm in controlled laboratory conditions [3, 4].

Heart rate variability (HRV) has been shown to reflect the autonomic nervous system's (ANS) control of the heart. HRV can be measured using standard frequency bands in power spectrum analysis of the HR signal [5]. Changes in HRV have been linked with cardiovascular disease states [6]. To better understand the diurnal variation in adverse cardiovascular events, we previously collected HR data at different circadian phases using a specialized time isolation protocol [7]. We showed a shift toward a parasympathetic dominant state during the habitual time of sleep, even when subjects were kept awake. There was also a shift toward a sympathetic dominant state during the habitual active period.

The aim of this study is to assess the ANS tone at different circadian phases and sleep-wake states, using a cardiorespiratory coherence (CRC) algorithm. CRC has previously been shown to reflect the balance between the sympathetic and parasympathetic modulation of the heart, by measuring the strength of linear coupling between heart rate and respiration. This provides one measure of respiratory sinus arrhythmia (RSA) [8]. RSA is a healthy heart arrhythmia, and has been shown to reflect the net balance between the sympathetic and parasympathetic tones [9]. Unlike conventional frequency analysis of HRV, CRC dynamically tracks the RSA as it moves in time/frequency. CRC does not assume fixed frequency bands, so it can function over a broad range of respiratory frequencies [8, 10]. CRC was shown to progressively increase from wakefulness to slow wave sleep (SWS), whereas it decreases during REM sleep in young adults [11, 12].

II. Methods

A. Subjects

Following ethics approval and informed consent, 9 healthy subjects (7 men, 2 women; mean age ± SD: 26.3 ± 4.6 years) were recruited to participate in a 5-day study. Exclusion criteria included any medical conditions, apnea/hypopnea index >10 events/h, drug, alcohol, and tobacco use, or night shift work. Women were excluded if they were using oral contraceptives or if they did not have a regular menstrual cycle. For at least 3 weeks prior to admission, subjects maintained a regular sleep-wake cycle according to their habitual sleep-wake schedule. Their compliance was verified by daily phone calls to the laboratory, a sleep-wake log, and actigraphic monitoring during the week before admission (AW-64, Mini Mitter Respironics, Bend, Or, USA).

B. Protocol

Subjects underwent an ultradian sleep-wake cycle (USW) procedure in a time isolation suite for 72 h (Fig. 1). The

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procedure began with an 8-h baseline sleep episode on the evening of Day 1. The baseline episode began at the subject's habitual bedtime, as reported in their sleep-wake log. Upon awakening, subjects began the USW procedure. This consisted of 60-min wake episodes in dim light (<10 lux) alternating with 60-min nap opportunities in total darkness. Throughout the USW procedure, participants remained in semi-recumbent position in bed, with low activity levels. Meals were replaced by balanced iso-caloric snacks administered during each wake episode. The USW is a procedure designed to quantify the circadian variation of a given parameter while minimizing the confounding influence of sleep deprivation, activity, food intake, and light levels on outcome measures. This specialized procedure is used to study the circadian variation of sleep and physiological parameters, as it allows sleep and waking to occur at a variety of circadian phases. The USW procedure was concluded with an *ad-libitum* nap episode on day 5.

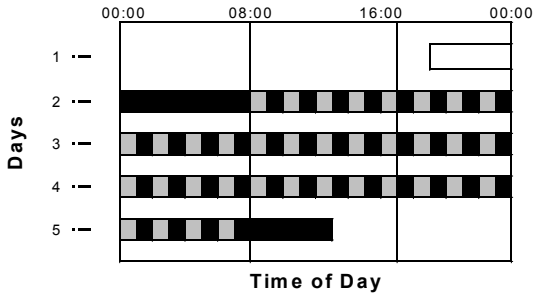

Fig. 1. USW procedure. Following an 8-h baseline sleep episode (Days 1-2), subjects began an USW cycle procedure for 72 h (Days 2-5). White bars represent waking episodes in 150 lux, grey bars represent waking episodes in dim light (<10 lux) and black bars represent sleep episodes in total darkness (<0.3 lux).

C. Measurement and Data Processing

Core body temperature (CBT) was continuously recorded throughout the experiment. A thermistor (Steri-Probe, Cincinnati Sub-Zero Products Inc., Cincinnati, OH, USA) was inserted 10 cm into the rectum and connected to an in-house data acquisition system. Measurements were taken every 15 s. Probe slips and malfunctions were removed by an ad-hoc program which excludes data < 36°C or > 38°C, or if the rate of change is > 0.2°C/min. Data were visually inspected before discarding values. CBT data were subsequently averaged into 1 min bins for further analysis.

Polysomnographic (PSG) sleep recording was performed throughout the USW procedure using custom hardware and the Harmonie system (Natus Medical Inc., Montreal, Canada). PSG recordings included a central and occipital electroencephalogram (EEG), electrooculogram (EOG), and submental electromyogram (EMG) sampled at 250 Hz, filtered using a high- and low-pass filter of 0.3 Hz and 35 Hz, respectively. PSG sleep recordings were visually scored according to standard criteria using 30-second epochs [13]. SWS was the amount of time spent in Stage 3 and 4 sleep.

Cardiorespiratory signals were recorded throughout the experiment using a LifeShirt (Vivometrics, Ventura, CA, USA). The LifeShirt contained built-in electrocardiogram (EKG) electrodes and dual band respiratory inductance plethysmography sensors. The EKG was recorded at 200 Hz, and thoracic and abdominal respiratory movement at 50 Hz. R-R intervals (RRIs) were extracted from the EKG using an automatic detection software. Ectopic beats and artifacts were removed by visual inspection of the RRI traces. Tidal volume was calculated based on a weighted sum of both thoracic and abdominal volumes (VivoLogic, Vivometrics, Ventura, CA, USA). The temporal derivative of the tidal volume was calculated and used as the respiration wave.

The HR and respiration data were prepared for coherence analysis. The RRIs were converted into a tachogram (HR series) and resampled onto an even 4 Hz grid using Berger's algorithm [14]. The respiratory frequency (f_R) was calculated from the respiration wave. The respiration wave was first split into 60-s windows with 50% overlap. The power spectrum of each window was then calculated using an FFT. The frequency of peak power was taken as f_R. Finally, f_R was median filtered (N = 25) to eliminate artifacts, and upsampled to 4 Hz using a repeater.

Cardiorespiratory coherence (CRC) was calculated for each data set. The algorithm first calculates the continuous wavelet transform of the tachogram and respiration waves using a complex Morlet basis over 96 scales. From the wavelet coefficients, it calculates the tachogram, respiration, and cross wavelet power in the joint time/frequency domain ($W_t^{TT}(s), W_t^{RR}(s), and\ W_t^{TR}(s)$, respectively, where s denotes scale). The powers are then smoothed in time with a Gaussian window ($e^{-t^2/2s^2}$) and in scale with a rectangular window (length 0.6 x scale). The algorithm then calculates the coherence estimator as:

$$\hat{C}_n^2(s) = \frac{\left|\left\langle W_t^{TR}(s)\cdot s^{-1}\right\rangle\right|^2}{\left\langle\left|W_t^{TT}(s)\right|\cdot s^{-1}\right\rangle\left\langle\left|W_t^{RR}(s)\right|\cdot s^{-1}\right\rangle}, \qquad (1)$$

where the angled brackets denote the smoothing operator. The coherence estimator is a 2D matrix of coherence values at each time and frequency. Finally, the algorithm extracts the coherence values at f_R at each point in time. The result is a 1D vector of time-varying CRC coherence values. CRC can range from 0 (no coherence) to 1 (perfect coherence).

CRC was used as a measure of ANS state. Higher coherence reflects stronger parasympathetic tone, while lower coherence reflects stronger sympathetic tone CRC measures ANS state using only RSA. For a more detailed description of the algorithm, see [10].

D. Analysis

The CBT minimum of each subject was assessed using a dual-harmonic regression model without serial correlated noise (courtesy of C.A. Czeisler, Brigham and Women's Hospital, Boston, MA, USA). CRC levels were assigned a circadian phase between 0° to 359.9° relative to the individual CBT minimum (set at 0°).

CRC levels across sleep stages were statistically compared

using a one-way within-subject ANOVA.For circadian analysis, CRC data were subsequently expressed as percentage differences from individual means throughout the 72-h USW protocol. They were then grouped into 30° bins, in order to obtain one complete 360° cycle (i.e. 24 h) during each sleep state. A nonlinear mixed model effect was applied to CRC data using the *nlmixed* SAS procedure (SAS Institute Inc., Cary, NC, USA) in order to assess circadian amplitude and phase during each sleep state. The model is described as:

$$y_{ijk} = b_0 + \beta_{1i} + b_1 \, State_{\,j} + A_j \cos\left(\tau_{ijk} - \phi_j\right) + \varepsilon_{ijk} \qquad (2)$$

where y_{ijk} denotes the k^{th} value, in the j^{th} state, for the i^{th} participant at circadian degree τ_{ijk} (360° = 24h). b_0 and β_{0i} describes the y-intercept (fixed and random effects, respectively). The state effect slope is described by b_1 (fixed effects). The circadian amplitude in each state is described by A_j. The corresponding phase of peak activity (acrophase) of the rhythm is described by ϕ_j. Significance level was set at $p \leq 0.05$. All results are expressed as mean ± SEM.

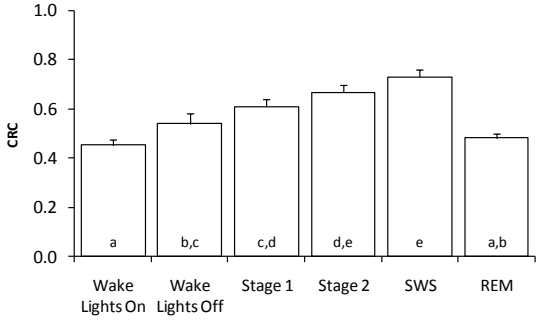

Fig. 2. CRC obtained during each sleep stage. Letters of each column are used to indicate significant differences between vigilance/sleep states, i.e., when two vigilance/sleep states have no common letter, they are significantly different ($p < 0.05$).Values are mean ± SEM.

III. Results

CRC showed significant changes between sleep states. It progressively increased with deepening of non-REM sleep (Fig. 2, $p < 0.0001$). CRC was significantly higher in Stage 2 and SWS than in wakefulness ($p \leq 0.0002$) and REM sleep ($p \leq 0.0001$). It was also significantly higher in SWS than in Stage 1 ($p = 0.0002$). CRC was significantly lower in REM sleep than in non-REM sleep ($p \leq 0.0002$), but not significantly different than wakefulness ($p \geq 0.13$).

A circadian variation of CRC was observed during all stages (wakefulness, both during lights on and off, Stage 1, Stage 2, SWS, and REM sleep; Fig. 3; $p \leq 0.02$). The CRC circadian amplitude was greater during REM sleep than in any other state ($p \leq 0.046$), except Stage 1 ($p = 0.09$). During Stage 2 and SWS, maximal CRC was observed around the habitual bedtime, whereas minimal CRC was observed at midday. Maximal CRC for wakefulness, REM sleep, and Stage 1 sleep was observed during the late afternoon and early evening, and minimal CRC was observed around the habitual time of awakening.

IV. Discussion & Conclusion

CRC progressively increased from wakefulness to SWS, whereas during REM sleep it decreased to levels comparable to those observed during wakefulness. Our results are consistent with prior effect size reported in young men using a different CRC technique [12]. It is interesting to note that simply turning the lights off and relaxing was associated with significant an increase in CRC. The ANS shift toward greater parasympathetic dominance during sleep is also in line with numerous studies looking at HRV [7, 15], direct sympathetic nerve recoding [16] or plasma catecholamine levels [17]. The increased parasympathetic dominance during sleep is potentially beneficial for the heart. On the other hand, the pronounced shift toward sympathetic dominance observed during REM sleep further supports the hypothesis that this sleep state represents a challenge for the cardiovascular system [16].

We observed a significant circadian rhythm of CRC during wakefulness and during each sleep stage. To our knowledge, no other study has examined the circadian variation of CRC during wakefulness or during sleep. Since it provides a measure of the ANS modulation of the heart [10], our findings could be compared to HR and HRV studies. During wakefulness, a circadian rhythm of HR has been shown using numerous experimental approaches, with HR decreasing during the habitual night, and increasing during the day [3, 4]. Our results are also consistent with HRV studies using various protocols such as constant routine [18], or forced desynchrony [19, 20] that showed a circadian rhythm in parasympathetic modulation of the heart, with parasympathetic dominance at night. Recently, we corroborated the circadian variation of the ANS balance during wakefulness and sleep using the USW procedure [7].

The circadian acrophase of CRC differed across sleep stages. During more active stages (wakefulness and REM sleep), the nadir of CRC was observed in the morning, around the habitual time of awakening. This finding indicates a shift toward greater sympathetic dominance in the morning, consistent with previous findings based on HRV [7, 21], plasma epinephrine [19], and pre-ejection period [22]. This ANS shift, added up to the sympathetic awakening response, could contribute to the increased risk of adverse cardiovascular events at that time of day. On the other hand, during non-REM sleep (Stage 2 and SWS), greater parasympathetic dominance was observed. This effect of sleep combined with the acrophase of CRC observed in the late evening and early night, underlines the protective effect of NREM sleep in the first half of the night [23].

Circadian distribution of sleep and sleep stages may be a limitation in this study. Though subjects were asked to sleep every other hour, sleep propensity and the amount of REM sleep vary across circadian phases [22]. Despite this limitation, our results support those based on different experimental approaches [3-5, 7, 11, 12, 15-22].

We have shown that CRC can be used to measure ANS state in conscious, spontaneously ventilated subjects. It can also discriminate between different sleep stages, and

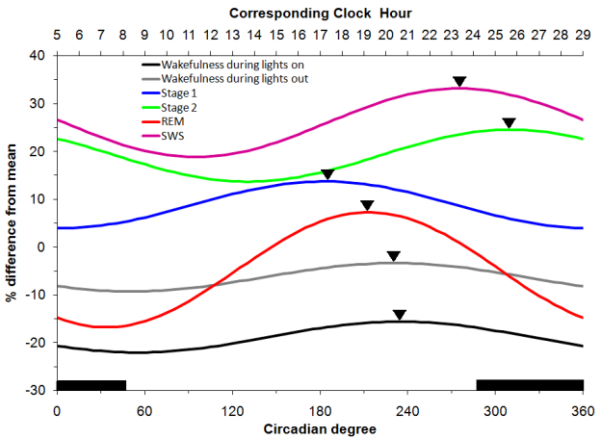

Fig.3. Circadian variation of CRC during different sleep stages. Modeled CRC levels are expressed as percentage difference from individual mean throughout the 72-h USW procedure. A non-linear mixed model effect was applied to individual subjects' 30°-binned data. The bottom x axis represents circadian phase relative to the CBT minimum (0° = 04:42). Black bars along the bottom x axis represent the projected time of habitual nocturnal sleep episodes and inverted triangles represent each acrophase.

responds to circadian variations in ANS tone. Previously, CRC had only been applied to mechanically ventilated patients during general anesthesia [6, 7].

Our findings suggest that circadian and sleep-wake dependant processes influence the ANS modulation of the heart. We observed increased sympathetic dominance in the morning during wakefulness and REM sleep, suggesting that these processes contribute to the higher incidence of adverse cardiovascular events at that time of day.

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