ELSEVIER

Clinical Neurophysiology 115 (2004) 19–28

www.elsevier.com/locate/clinph

Estimating cardiac autonomic activity during sleep: impedance cardiography, spectral analysis, and Poincaré plots

Helen J. Burgess*, Plamen D. Penev, Renate Schneider, Eve Van Cauter

Research Laboratory on Sleep, Chronobiology and Neuroendocrinology, Section of Endocrinology, Department of Medicine, University of Chicago, Chicago, IL, USA

Accepted 29 August 2003

Abstract

Objective: To compare noninvasive measures of cardiac autonomic activity during sleep.

Methods: The absolute and normalized (n.u.) high and low frequency peaks from the spectral analysis of R-R intervals (HF, LF, HFn.u., LFn.u.), LF/HF ratio, pre-ejection period (PEP) from impedance cardiography, and the autocorrelation coefficient (rRR) as illustrated in Poincaré plots were measured during night-time sleep in 9 young healthy subjects. Heart rate and blood pressure were also recorded.

Results: Heart rate was significantly associated with cardiac sympathetic activity (PEP, average $r = -0.46$), but not with cardiac parasympathetic activity (HF, average $r = -0.17$). rRR was significantly associated with heart rate (average $r = 0.41$), and LF/HF (average $r = 0.69$), but not with PEP or HF. From NREM to REM sleep, heart rate, LFn.u., LF and rRR significantly increased, HFn.u. significantly decreased, LF/HF showed an increasing trend ($P = 0.07$) and PEP showed a decreasing trend ($P = 0.06$). Blood pressure and HF were highly variable without significant changes from NREM to REM sleep.

Conclusions: Cardiac parasympathetic activity (HF) does not vary greatly between sleep stages. Cardiac sympathetic activity (PEP) decreases linearly during sleep. rRR and LF/HF can track sympathovagal changes during sleep, but cannot differentiate between changes in cardiac parasympathetic and sympathetic activity. The relative advantages and disadvantages of the different measures are discussed.

Keywords: Autonomic; Cardiac; Heart; Sleep; Sympathetic; Parasympathetic

1. Introduction

Estimating cardiac autonomic activity during sleep has emerged as a new focus of interest, due to the clinical importance of assessing the impact of sleep on cardiovascular activity. Cardiac autonomic activity in humans is necessarily assessed with noninvasive techniques. A number of sleep studies have used a measure derived from Poincaré plots of consecutive R-R intervals, the autocorrelation coefficient rRR, to examine 'sympathovagal' balance (Schechtman et al., 1992; Otzenberger et al., 1997, 1998; Spiegel et al., 1999; Ehrhart et al., 2000). These studies have consistently shown that rRR oscillates in synchrony with NREM-REM sleep cycles (Schechtman et al., 1992; Otzenberger et al., 1997, 1998; Ehrhart et al., 2000). The physiological significance of the rRR index remains, however, unclear as this index has not been carefully validated by pharmacological blockades. In other studies (e.g. Trinder et al., 2001), cardiac autonomic activity during sleep was estimated using extensively validated measures; i.e. high frequency power from the spectral analysis of R-R intervals and pre-ejection period from impedance cardiography, that are purported to specifically reflect cardiac parasympathetic (vagal) and cardiac sympathetic activity, respectively. We performed a direct comparison of these measures in order to explore the potential physiological significance of changes in rRR during sleep, and to present a general overview of these measures.

While many measures of autonomic activity can be derived from the electrocardiograph (ECG), the most commonly used are the high frequency (HF) and low frequency (LF) measures. These measures are derived from the spectral analysis of R-R intervals that are typically

* Corresponding author. Biological Rhythms Research Laboratory, Rush University Medical Center, 1645 West Jackson Boulevard, Suite 425, Chicago, IL 60612, USA. Tel.: +1-312-563-4785; fax: +1-312-563-4900.
E-mail address: hburgess@rush.edu (H.J. Burgess).

1388-2457/$30.00
doi:10.1016/S1388-2457(03)00312-2

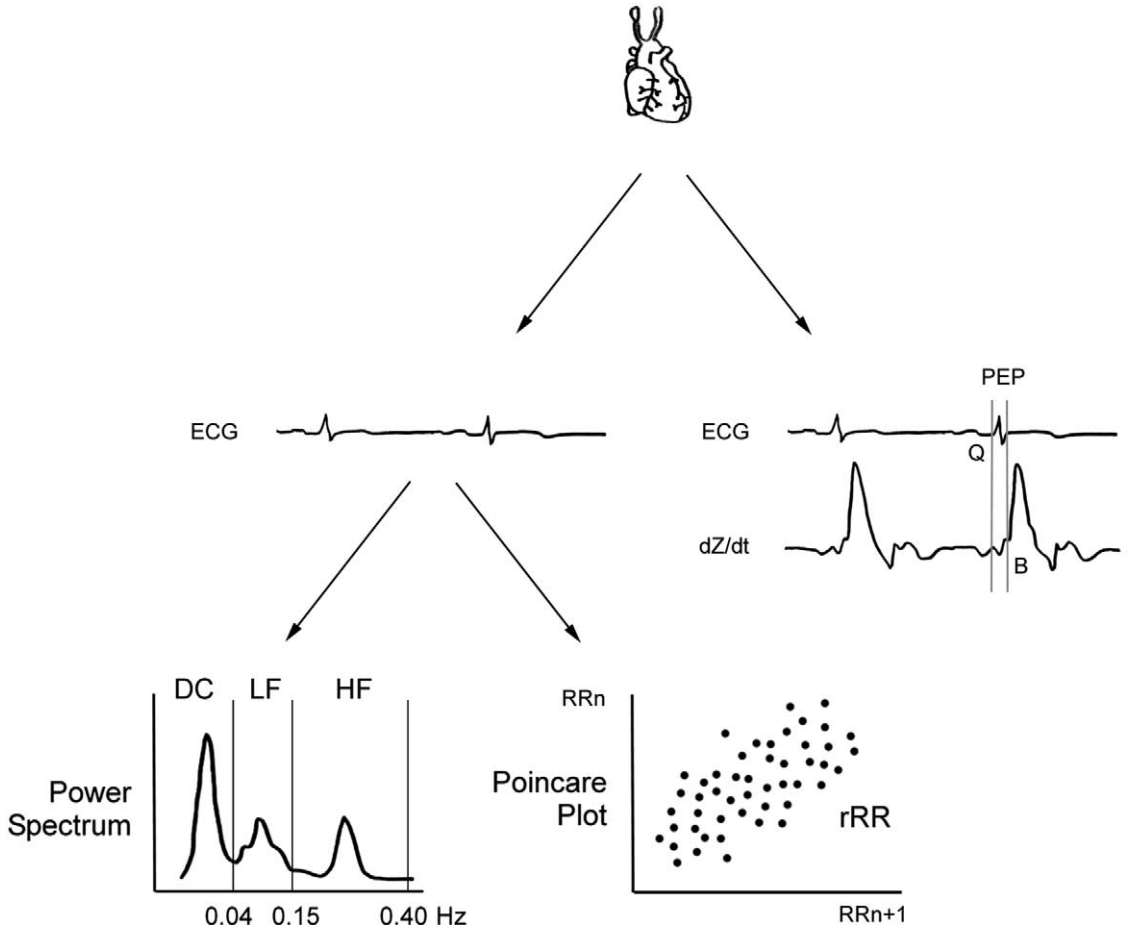

Fig. 1. A schematic diagram illustrating the derivation of LF, HF, rRR and PEP. A period of stable R-R intervals in the ECG is selected. LF and HF are derived from a power spectrum generated by the spectral analysis of a series of R-R intervals, in turn derived from the ECG (bottom left). The area in set frequency bands in the power spectrum represents the magnitude of LF or HF (0.04–0.15 and 0.15–0.40 Hz, respectively). As the absolute power in these bands can vary widely within and between individuals, the power of LF and HF can be 'normalized' by dividing the power in each band by the total power (0.04–0.40 Hz). The resulting LFn.u. and HFn.u. vary between 0 and 1. Poincaré plots (bottom right) are calculated by plotting each R-R interval against the previous R-R interval. From these data points a correlation coefficient, rRR, is calculated. PEP (middle right) is derived by comparing the ECG signal to d*Z*/d*t* (the change in impedance to an externally generated electrical signal across time). PEP is the time interval between the Q wave in the ECG and B point on the d*Z*/d*t* signal.

selected from a 2–5 min period of stable ECG activity (see reviews Ori et al., 1992; Kamath and Fallen, 1993). Briefly, the spectral analysis produces a power spectrum ranging from 0 to ~0.40 Hz (Fig. 1, bottom left). The HF measure is usually calculated as the area under the curve within the range of 0.15–0.40 Hz, and the LF measure is often calculated as the area under the curve within the range of 0.04–0.15 Hz (see review by Berntson et al., 1997). As the power in these bands can vary widely within and between individuals, the absolute power of LF and HF can be 'normalized' by dividing the power in each band by the total power minus DC noise (0.04–0.40 Hz). Thus the resulting 'LFn.u.' and 'HFn.u.' (n.u. = normalized units) can theoretically vary between 0 and 1 and HFn.u. + LFn.u. = 1.

HF is a well-accepted noninvasive measure of cardiac parasympathetic activity in humans. HF is believed to largely reflect the degree of respiratory sinus arrhythmia in the ECG; R-R intervals increase during expiration and decrease during inspiration. Detailed studies have shown that only parasympathetic fibers contribute to the generation of respiratory sinus arrhythmia (see Berntson et al., 1993, 1997). Indeed, pharmacological blockade studies have shown that HF specifically reflects cardiac parasympathetic activity (e.g. Akselrod et al., 1981; Pomeranz et al., 1985; Cacioppo et al., 1994). Others have suggested that HFn.u. is also a relatively pure measure of cardiac parasympathetic activity (Malliani et al., 1991, 1994), although as part of its derivation, HFn.u. will include power in the LF band, which as described below, may mean that HFn.u. reflects both sympathetic and parasympathetic activity. Much of the early work that explored sleep and circadian influences on cardiac parasympathetic activity only reported HFn.u. (e.g. Burgess et al., 1996, 1997).

Pharmacological blockade studies have also revealed that contrary to some reports (Malliani et al., 1991, 1994), LF is not a specific measure of cardiac sympathetic activity

(e.g. Akselrod et al., 1981; Pomeranz et al., 1985). Instead, the general consensus is that LF and LFn.u. most likely reflect a mix of both parasympathetic and sympathetic influences (see review by Berntson et al., 1997). Despite this, the ease of calculating LF (particularly if HF is also being calculated) has led to its continued use by some as a marker of cardiac sympathetic activity. The LF/HF ratio is often calculated as a marker of 'sympathovagal balance' (Malliani et al., 1991, 1994).

The best validated measure of cardiac sympathetic activity is pre-ejection period (PEP). PEP (Fig. 1, middle right) is derived from impedance cardiography (Sherwood et al., 1990). In brief, a small harmless externally generated electrical signal is passed across the chest and the change in impedance across time (dZ/dt) is measured. PEP is the time interval between the Q wave on the ECG signal and the 'B point' on the dZ/dt signal. Physiologically, PEP represents the time interval in which the left ventricle, that is largely innervated by β-adrenergic sympathetic fibers, contracts while both the aortic and mitral valves are closed. Systematic pharmacological blockade studies have supported the use of PEP as a specific measure of cardiac sympathetic activity (e.g. Cacioppo et al., 1994; Schachinger et al., 2001). As sympathetic activity increases, PEP shortens.

Poincaré plots (Fig. 1, bottom right) are calculated by plotting each R-R interval, against the following R-R interval (R-Rn vs. R-Rn_{+1}). From these data points, the correlation coefficient rRR can be calculated. This coefficient can theoretically vary between 0 and 1. To date, only two studies have examined Poincaré plots during pharmacological blockades (Zemaityte et al., 1984; Kamen et al., 1996). While Kamen et al. suggested that measures derived from the plots are relatively pure measures of cardiac parasympathetic activity, neither study specifically assessed rRR. Thus the validity of rRR as a specific measure of cardiac parasympathetic activity remains uncertain. If rRR is a specific measure of cardiac parasympathetic activity then we expect it to correlate highly with HF but not with PEP. Previous reports found a weak correlation between rRR and HF during sleep (mean $r = -0.19$, Otzenberger et al., 1998), but no study has yet examined the relationship between rRR and PEP.

Thus the current study had two aims. The first was to examine the relationship between rRR and specific measures of cardiac autonomic activity, HF and PEP, in order to assess the potential physiological significance of rRR. The second broader aim was to examine the relationships between HFn.u., LFn.u., LF/HF, PEP, and rRR during night-time sleep episodes and in doing so, to present for the first time, a general overview of the advantages and disadvantages of these separate measures, which are often used to evaluate changes in cardiac autonomic control during sleep.

2. Methods

2.1. Subjects

Nine young males (mean ± SE; age 23.3 ± 5.5 years, body mass index 21.6 ± 2.6 kg/m^2) participated. All subjects were healthy with no clinically significant neurological, psychiatric and sleep disorders. They also had no chronic medical conditions requiring active treatment. All subjects reported drinking less than 10 standard alcoholic drinks per week, were not night workers and had not traveled across time zones in the previous month. The study protocol was approved by the University of Chicago's Institutional Review Board. All subjects gave written informed consent prior to their participation. Subjects received financial compensation for their time.

2.2. Design

All experimental sessions were conducted in the Research Laboratory on Sleep, Chronobiology and Neuroendocrinology (RL-SCN) at the University of Chicago. Participants had at least one adaptation night prior to their experimental night, and these were separated by at least 24 h.

2.3. Procedures

2.3.1. General laboratory procedures

On each night participants arrived several hours prior to their usual bedtime (bedtime varied between ~22:00–23:30 h). Subjects toileted, had the equipment attached (see below), and were put to bed in individual temperature-controlled bedrooms. At their usual bedtime the lights were turned out and they were permitted to sleep. All subjects were left undisturbed until their normal wake up time.

2.3.2. Assessment of sleep-wake state

Each subject's sleep-wake state was assessed by a central (C3-A2) and occipital (O1-A2) electroencephalogram (EEG), an electro-oculogram (EOG; left and right outer canthi displaced vertically), and electromyogram (EMG, submental) according to standardized criteria (Rechtschaffen and Kales, 1968). An Alice-4 HOST sleep analysis system (Respironics, Pittsburgh, PA, USA) was used to collect and analyze the data, which was scored by two independent raters in 30 s epochs according to standard procedures (Rechtschaffen and Kales, 1968).

2.3.3. Impedance cardiography: assessment of HR and PEP

An electrocardiogram (ECG) was obtained from disposable pregelled Ag/AgCl ECG spot electrodes (Meditrace, USA) that were placed at the jugular notch of the sternum, 4 cm under the left nipple and the right lateral side. The electrodes were connected to a VU-AMS device (version 4.6, TD-FPP, Vrije Universiteit, Amsterdam,

The Netherlands). The ECG signal was filtered with a low pass software filter of 17 Hz and then amplified by the VU-AMS device using an amplifier with a time constant of 0.3 s and 1 MΩ impedance. Each R-peak was detected with a level detector with automatic level adjustment (Thakor et al., 1983). From the R-peak time series an average value for heart rate was obtained for each 30 s.

The VU-AMS also determined pre-ejection period (PEP) via impedance cardiography. In order to measure PEP, a 350 μA current at 50 kHz was passed through the body via 'current' electrodes on the base of the neck over vertebrae C3/C4 and on the back over vertebrae T8/T9. Two 'recording' electrodes, on the jugular notch and xiphoid process of the sternum, measured the resulting impedance (Z_0), from which the change in impedance with time (dZ/dt) was derived. The dZ/dt signal was sampled at 250 Hz, and time locked to the R wave to enable 30 s ensemble averaging of the dZ/dt signal. Thus for each 30 s period, PEP was later determined off-line as the time period between the R wave on the ECG signal and the upstroke (B point) on the ensemble averaged dZ/dt signal. Reliability and validity of the VU-AMS device is described elsewhere (de Geus et al., 1995; Willemsen et al., 1996).

2.3.4. Spectral analysis of heart rate variability: assessment of HF and LF

To measure HF and LF, 2 min periods of cardiac beat-to-beat intervals (determined with the VU-AMS) were selected for spectral analysis (see below). Frequency domain analysis of the inter-beat intervals was calculated by the CARSPAN program (ProGAMMA, The Netherlands) that is based on sparse discrete Fourier transformation and produces a spectrum from 0.01 to 0.40 Hz. The spectrum is based on a series of equidistant samples representing HR, obtained from low pass filtering of the R-wave series as unit pulses. HF was calculated as the power in the range of 0.15–0.40 Hz. LF was calculated as the power in the range of 0.04–0.15 Hz. HFn.u. was calculated as HF divided by the total power (0.04–0.40 Hz). LFn.u. was calculated as LF divided by the total power (0.04–0.40 Hz). The LF/HF ratio was also calculated.

2.3.5. Poincaré plots of heart rate variability: assessment of rRR

A Mini-logger system (Mini-Mitter Co. Inc., Sunriver, OR, USA) recorded R-R intervals via a Polar chest belt (Polar heart rate monitor, Polar cic inc., Port Washington, NY.). This system recorded the interbeat intervals after the system amplified the ECG signal and each R-peak was detected with a level detector. rRR was calculated in 2 min periods (see below for how they were selected) by calculating the correlation between each R-R interval (R-Rn) and the following R-R interval (R-Rn_{+1}). A high rRR indicates lower beat-to-beat variability, while a low rRR reflects higher beat-to-beat variability. R-R intervals $>$ 1500 ms or $<$ 400 ms, or consecutive R-R intervals with an absolute change greater than 150 ms were discarded from further analysis (Spiegel et al., 1999).

2.3.6. Assessment of blood pressures

A portable Accutracker II (Suntech Medical Instruments, USA) was used to measure diastolic and systolic blood pressures (DBP, SBP) every 10 min. This system used a cuff placed over the upper arm, that inflated every 10 min, and 3 disposable pregelled Ag/AgCl ECG spot electrodes (Meditrace, USA). Two of the electrodes were placed just below the two ICG electrodes on the left and right side of the rib cage and the third was placed under the middle portion of the right clavicle. The Accutracker system recorded the blood pressures and automatically scored as invalid SBP $>$ 220 or $<$ 80 mmHg, or if there was a change of $>$ 50 mmHg compared to the preceding measurement. Similarly, DBP values were scored as invalid if DBP $>$ 130 or $<$ 40 mmHg or if there was a change of $>$ 40 mmHg between consecutive measurements.

2.4. Data analysis

The following sleep variables were calculated for each subject: percentage of wake, Stage 1, Stage 2, Stage 3 + 4 (slow wave sleep, SWS), REM sleep and sleep efficiency. The cardiovascular data collected from sleep onset (first of 3 consecutive 30 s epochs of scored NREM sleep Stage 1 or 2) to final awakening were analyzed. Thirty-second epochs of all the data were individually inspected and epochs that contained movement artifact were discarded. The raw data of each of the cardiac variables were binned into 10 min bins which began 5 min before and ended 5 min after each blood pressure reading. In this way all of the cardiac variables were aligned in time with each other and with the blood pressure recordings.

2.4.1. Statistical analysis

In order to assess changes in the measures between NREM and REM sleep, each 10 min bin that was scored entirely as NREM or REM sleep was identified. For each variable, these bins were then averaged within each subject to produce a mean value for NREM and REM sleep. A paired t test identified any significant change in each variable between NREM and REM sleep.

To investigate the relationships between the cardiac variables during the sleep period, we calculated Pearson product moment correlations based on the z scores derived for each measure from each subject. The correlations were first calculated for each individual, based on the 10 min bins. The correlations were then converted to z scores using Fisher z-transformations. These z scores were averaged across the group, and these averages were then converted back to group correlation coefficient scores. To estimate the statistical significance, a t test was conducted to determine if the mean correlation was significantly different from zero.

Table 1
Mean and standard errors (SE) of the percentage of each sleep variable during the experimental night

	Mean	SE
Wake	5.75	1.18
Stage 1 NREM sleep	5.92	0.95
Stage 2 NREM sleep	48.60	3.33
Slow wave sleep	19.94	2.62
REM sleep	19.58	1.42

Percentages were calculated as a proportion of time spent in bed from lights out to final morning awakening.

3. Results

All subjects slept well with mean sleep efficiency of 94.4 ± 4.2% (SE) and normal sleep stage distribution (see Table 1). Fig. 2 shows the pattern of change in HR, PEP, HFn.u., rRR, SBP and DBP across time, during the sleep period of an individual subject. Fig. 3 shows the changes in total spectral power, HF, LF, HFn.u., LFn.u. and LF/HF during the same sleep period. We did not calculate mean profiles for the group, as this would have obscured the NREM to REM sleep transitions. The profiles of HR and PEP exhibited underlying linear trends for sleep onset to morning awakening (e.g. see Fig. 2) that partly masked the changes between NREM and REM sleep (e.g. Burgess et al., 2001a; Trinder et al., 2001). Thus for these two variables we fit linear regressions to each individual profile and then analyzed the residuals from these regressions.

3.1. Correlations between cardiovascular variables

The average correlations from all 9 subjects are shown in Table 2. As expected HR and SBP were significantly positively associated: as HR increased, SBP increased. HR was significantly negatively correlated with PEP, but was not significantly correlated with HF (although the correlation was in the expected direction). Thus, an increase in HR during sleep was associated with an increase in cardiac sympathetic activity (shortening of PEP), but not with any significant changes in HF. HF and PEP were not significantly correlated. The autocorrelation coefficient, rRR was significantly positively associated with HR (Fig. 2), but was not significantly associated with PEP or HF.

It is clear from Table 2 and Fig. 3 that as a function of how they are calculated, HFn.u. and LFn.u. are mirror images of each other. As HFn.u. increases, LFn.u. must decrease and vice versa. Interestingly, HF and HFn.u. were not associated, although LF and LFn.u. were significantly positively associated with each other. Thus changes in HFn.u. were largely due to the changes in LF. Additionally, LF/HF was more strongly associated with LF than HF. These results are probably due to the LF band generally

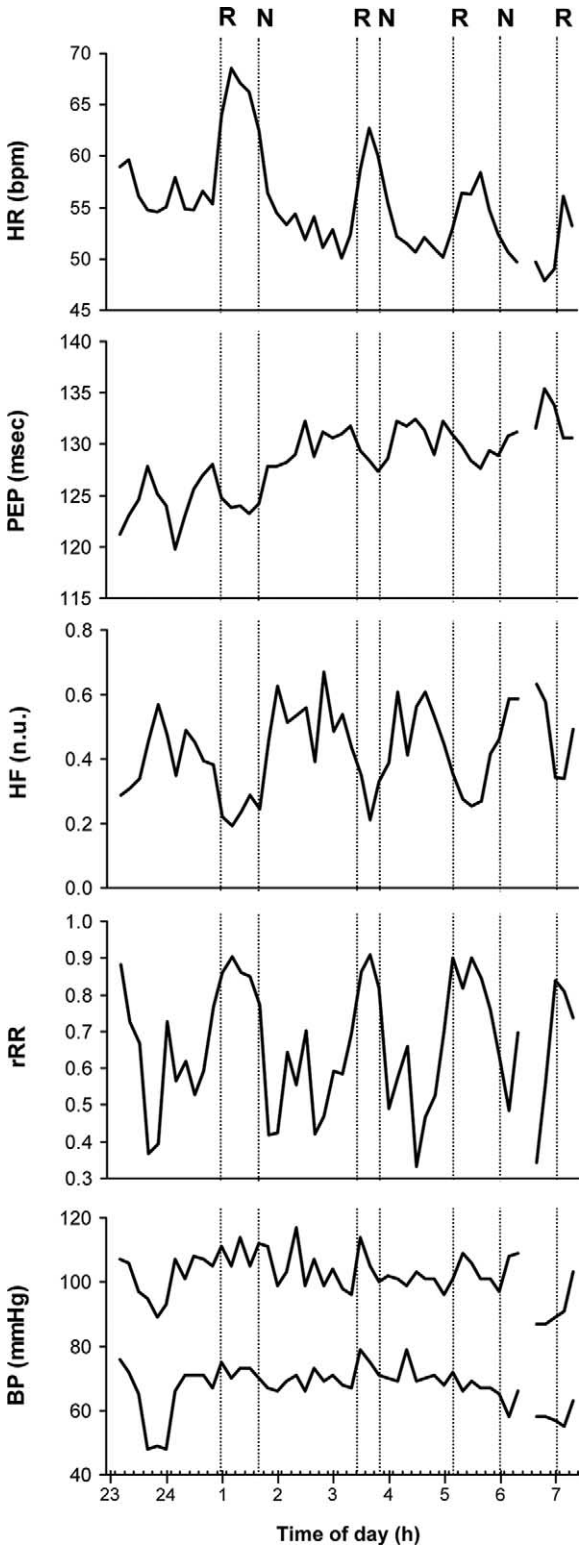

Fig. 2. The time course of HR (bpm), PEP (ms), HFn.u., rRR and SBP (top line, mmHg), and DBP (bottom line, mmHg), during the sleep episode of an individual subject. The data are from the first onset of NREM sleep. Subsequent onsets of REM and NREM sleep are represented by the dashed vertical lines labeled R and N, respectively. The data are in 10 min bins. The missing data in the last NREM period is due to the fact that data at this time were confounded by movement. Changes in the variables prior to the onset of REM sleep can be seen.

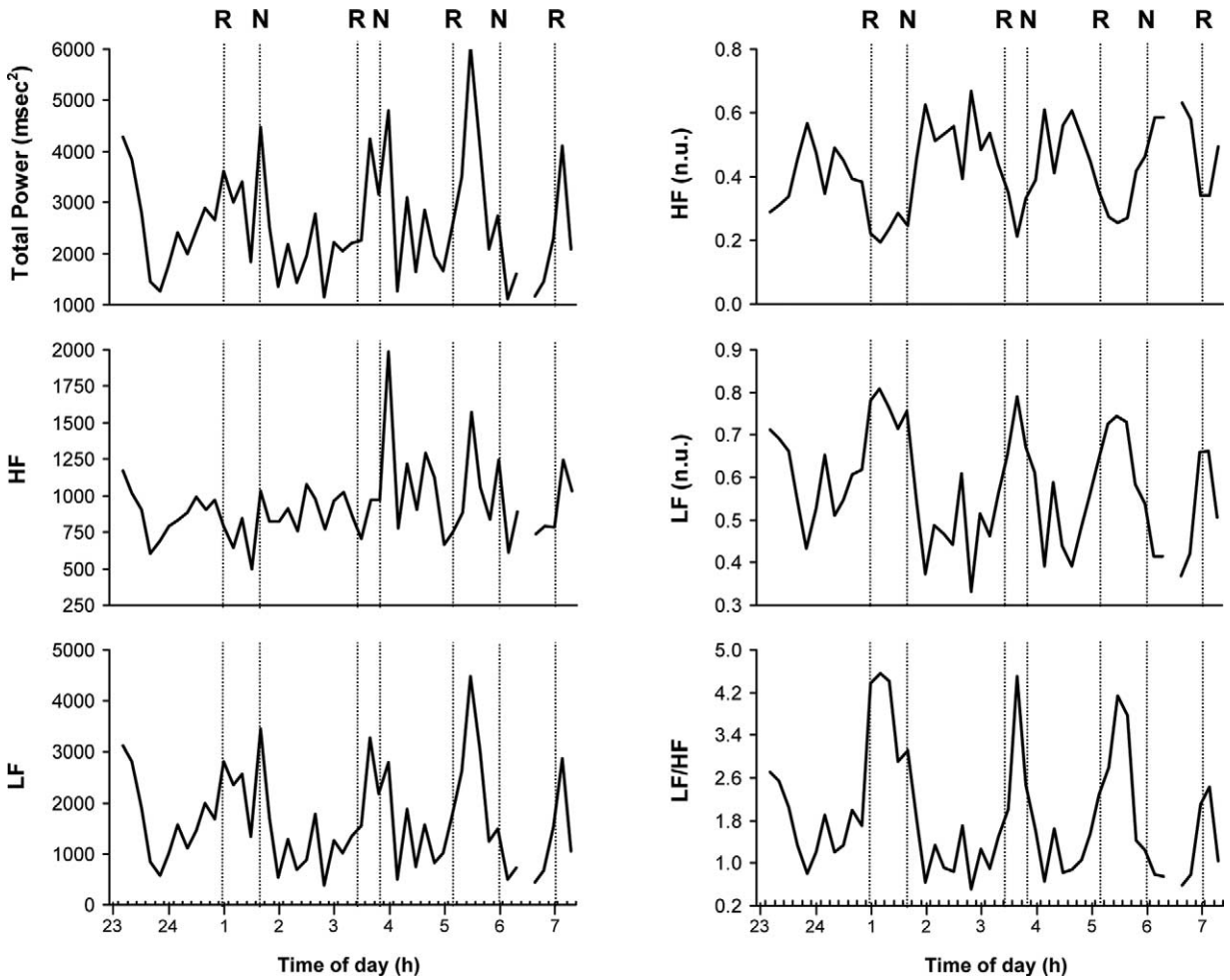

Fig. 3. The time course of total spectral power, HF, LF, HFn.u., LFn.u. and LF/HF during the sleep episode of an individual subject. The data are from the first onset of NREM sleep. Subsequent onsets of REM and NREM sleep are represented by the dashed vertical lines labeled R and N, respectively. The data are in 10 min bins. The missing data in the last NREM period is due to the fact that data at this time were confounded by movement. Changes in the variables prior to the onset of REM sleep can be seen.

containing more power than the HF band. Indeed in a post hoc analysis we determined that LF was more highly correlated with total power than HF (average $r = 0.97$, $P < 0.05$; average $r = 0.83$, $P < 0.05$, respectively).

3.2. NREM and REM sleep

The mean values of the variables in NREM and REM sleep are presented in Table 3. The average HR in

Table 2
Mean Pearson product moment correlations between the cardiac variables during the entire sleep phase

	HR	PEP	HF	HFn.u.	LF	LFn.u.	LF/HF	rRR	DBP	SBP
HR	–									
PEP	−0.46*	–								
HF	−0.17	0.06	–							
HFn.u.	−0.54*	0.31*	0.03	–						
LF	0.26	−0.22*	0.66*	−0.48	–					
LFn.u.	0.54*	−0.31*	−0.03	−1.00*	0.67*	–				
LF/HF	0.58*	−0.31*	−0.09	−0.93*	0.66*	0.93*	–			
rRR	0.41*	−0.15	0.13	−0.77*	0.56*	0.77*	0.69*	–		
DBP	0.03	0.06	0.07	−0.02	0.03	0.02	0.05	0.08	–	
SBP	0.38*	−0.12	0.05	−0.13	0.09	0.13	0.17	0.22	0.47*	–

The correlations are for $n = 9$. *$P < 0.01$.

Table 3
Mean values (±SE) during NREM and REM sleep, relative change from NREM to REM sleep and corresponding effect size

	NREM	REM	Relative change	Effect size
HR	60.29 ± 3.13	62.84 ± 2.26	+ < 5% ($P < 0.05$)	0.33
PEP	122.09 ± 4.49	121.20 ± 4.97	− < 1% (n.s.)	0.07
HF	1194 ± 223	1643 ± 523	+38% (n.s.)	0.39
HFn.u.	0.50 ± 0.06	0.35 ± 0.05	−30% ($P < 0.05$)	0.94
LF	1528 ± 271	2821 ± 401	+85% ($P < 0.05$)	1.34
LFn.u.	0.50 ± 0.06	0.65 ± 0.05	+30% ($P < 0.05$)	0.94
LF/HF	1.74 ± 0.51	2.70 ± 0.56	+57% ($P = 0.07$)	0.64
rRR	0.57 ± 0.07	0.78 ± 0.03	+37% ($P < 0.05$)	1.35

Note that here HR and PEP have not been corrected for the underlying linear trend (see Section 3). n.s., not significant.

REM sleep was 3.93 bpm (±1.07 (SE)) higher than in NREM sleep ($P < 0.01$, with linear trend removed). The average PEP in REM sleep was 4.69 ms (±2.90) shorter than in NREM sleep ($P = 0.06$, with linear trend removed), reflecting a trend for increasing cardiac sympathetic activity in REM sleep. HFn.u. significantly decreased from NREM to REM sleep by 0.16 ± 0.03 n.u. ($P < 0.01$), while HF did not significantly change between the two states ($P = 0.21$). As expected, LFn.u. significantly increased from NREM to REM sleep by 0.16 ± 0.03 n.u. ($P < 0.01$), and LF showed the same significant effect with an increase in power of 1293 ± 351 ms^2 ($P < 0.01$). Total power also significantly increased from NREM (2722 ± 369 ms^2) to REM sleep (4465 ± 844 ms^2, $P < 0.05$). LF/HF showed an increase of 0.97 ± 0.49 n.u. from NREM to REM sleep ($P = 0.07$). Finally, rRR significantly increased from NREM sleep to REM sleep by 0.21 ± 0.06 ($P = 0.01$). Blood pressure tended to be higher during REM than during NREM sleep, but the differences were not significant (DBP: NREM 60.6 ± 1.5, REM 62.9 ± 2.1 mmHg, $P = 0.15$; SBP: NREM 103.2 ± 2.0, REM 105.0 ± 2.5 mmHg, $P = 0.48$).

In Table 3 we also summarize for each cardiac variable the relative change from NREM to REM sleep and the corresponding effect size. This analysis shows that PEP (not corrected for the linear trend across the night) decreased very little in relation to the variability of the measurements from NREM to REM sleep. In contrast, rRR and LF both increased substantially from NREM to REM sleep, and therefore were much more sensitive (although perhaps less specific) markers of changes in cardiac autonomic control across sleep stages than PEP.

4. Discussion

Our results suggest that rRR can track sympathovagal changes during sleep but is not a specific measure of either cardiac parasympathetic or sympathetic activity. We found a weak relationship between rRR and HF ($r = 0.13$), of similar magnitude as the correlation reported in the single other study that correlated the two measures (average $r = -0.19$, Otzenberger et al., 1998). It is possible that this low correlation is due to the generally elevated cardiac parasympathetic tone during sleep, and this reduced our ability (and others) to find a significant correlation between rRR and HF (restriction of range effect). For the first time we compared rRR with PEP and found they also were only weakly related ($r = -0.15$). This suggests that at least during sleep, rRR is not immediately or greatly influenced by cardiac sympathetic activity alone. However, the weak correlation between rRR and PEP may also be due to the fact that the changes in PEP between NREM and REM sleep were fairly small (again restriction of range effect). Others have also reported on average about a 1 ms change in PEP from NREM to REM sleep (Trinder et al., 2001). Thus further work needs to investigate rRR during wakefulness and sleep in order to examine rRR when parasympathetic activity is less elevated and sympathetic activity is more variable. To fully determine the autonomic contributions to rRR, this measure needs to be examined during systematic pharmacological blockades.

The other correlations amongst the autonomic variables are consistent with the interpretation of HF and PEP as specific measures of cardiac parasympathetic and sympathetic activity respectively (e.g. Akselrod et al., 1981; Pomeranz et al., 1985; Cacioppo et al., 1994; Schachinger et al., 2001). HF and PEP correlated with HR in the expected direction, although the correlation between HF and HR was not significant. HF and PEP were not significantly correlated with each other, suggesting that the two autonomic branches did not change reciprocally. This has also been seen in individual responses to psychological stress: one branch can change its activity with little change in the other branch (Berntson et al., 1994). The patterns of change in cardiac parasympathetic and sympathetic activity prior to and during sleep also highlight that the two branches can vary somewhat independently of each other. In earlier work using HFn.u., cardiac parasympathetic activity was found to increase prior to normal sleep onset time, apparently as part of a circadian influence (Burgess et al., 1997). Later work using HF in forced desynchrony protocols found a similar pattern (Umali et al., 2000). Importantly, this apparent increase in cardiac parasympathetic activity prior to sleep was not simply due to the concomitant increase in melatonin (Burgess et al., 2001b; Harris et al., 2001). In contrast to the presleep increase in parasympathetic activity, several studies have shown that cardiac sympathetic activity (measured with PEP) does not decrease, until after the onset of sleep (Burgess et al., 1997, 1999; Trinder et al., 2001).

The changes we observed between NREM and REM sleep are similar to previous reports. We saw, as others have, a significant decrease in HFn.u. and increase in LFn.u. during REM sleep (e.g. Van De Borne et al., 1994; Ferini-Strambi et al., 1997; Trinder et al., 2001). However, we did not observe any significant change in HF, consistent

with previous reports that HF does not always significantly decrease from NREM to REM sleep (Umali et al., 2000; Brandenberger et al., 2001; Trinder et al., 2001; Ako et al., 2003). Thus the significant changes in HFn.u. were likely to be due to the significant changes in LF, and thus probably reflect a mix of sympathetic and parasympathetic activity. We also found a close to significant increase in the LF/HF ratio from NREM to REM sleep, similar to what others have previously reported (e.g. Baharav et al., 1995; Bonnet and Arand, 1997; Ferini-Strambi et al., 1997; Elsenbruch et al., 1999; Ehrhart et al., 2000; Ako et al., 2003). Four earlier studies also found an increase in rRR during REM sleep as compared to NREM sleep (Schechtman et al., 1992; Otzenberger et al., 1997, 1998; Ehrhart et al., 2000). These changes are clearly seen in an individual subject's data shown in Figs. 2 and 3. Changes in all of these variables, prior to the onset of REM sleep is also clear. That the autonomic changes in REM sleep can be seen to occur before the change in scored sleep (Cajochen et al., 1994; Bonnet and Arand, 1997; Otzenberger et al., 1998; Burgess et al., 2001a) is likely in part due to the insensitivity of standard sleep scoring to the subtle changes in central nervous system activity (Rechtschaffen and Kales, 1968).

While increases in BP are well known correlates of REM sleep, we did not observe a significant increase in SBP or DBP in REM sleep, as compared to NREM sleep. Our analytical method averaged all REM sleep, whether phasic or tonic together. BP is typically highly variable in REM sleep, with low levels during tonic REM sleep and abrupt increases during phasic REM sleep (Mancia and Zanchetti, 1980). Thus our analysis probably averaged out these changes, such that NREM and REM sleep were not significantly different. Similarly, because of our averaging method we were not able to determine cardiovascular differences between NREM Stage 1 and 2 versus SWS.

It is possible that by measuring BP with an inflating cuff every 10 min, we disturbed the subjects' sleep and thus altered their autonomic activity. To investigate this possibility, we compared the percentages of wake and stage 1 sleep in this study with previous studies of healthy young subjects who slept without an inflating cuff (e.g. Burgess et al., 1999, 2001a). We found that our current subjects had similar amounts or even less wake or stage 1 sleep than in our previous studies. Nonetheless, it is still possible that if the inflation of the cuff led to brief arousals, such sleep changes would not be detected with traditional Rechtschaffen and Kales sleep scoring. We also looked for a direct effect of the BP measurement on the cardiovascular variables. Within each subject we compared the first 2 min with the last 2 min of each 10 min bin (paired t test). In every subject we found no significant effect of the BP measurement on any of cardiovascular variables. This result, combined with the fact that we observed similar changes in these variables to what others have reported (see above), suggests that if the cuff inflation affected autonomic activity, these changes were much smaller than the immediate effect of night-time sleep.

Whilst HF and PEP are specific measures of parasympathetic and sympathetic activity, respectively, these measures both have associated confounds. HF can be confounded by variations in respiratory activity. Specifically, during fast breathing, respiratory sinus arrhythmia (and thus HF which quantifies respiratory sinus arrhythmia) may underestimate parasympathetic activity (Berntson et al., 1997). However, in previous sleep studies of normal young healthy subjects, the relationship between respiratory rate and HFn.u. (HF was not reported) was not particularly strong (e.g. average $r = -0.19$, Burgess et al., 1999, average $r = -0.04$, Ehrhart et al., 2000), and a recent paper shows that respiratory rate does not vary greatly during sleep (Trinder et al., 2001, which is why we did not measure it here). Indeed, the respiratory changes during sleep appear to confound the interpretation of HF to a lesser degree than the larger changes in respiration observed during waking psychophysiological experiments (the field where much of the validation of HF has occurred).

PEP may be confounded by variations in BP. If BP is high, then it is possible that PEP may be lengthened due to the fact that greater pressure must be built up before the valve is forced open. Thus, with higher BPs, the lengthening of PEP may reflect the increase in time required to overcome this external pressure, rather than a decrease in cardiac sympathetic activity. Again however, in sleep and circadian studies we have not found the BP changes to be strongly related to PEP (e.g. $r = 0.26$ in Burgess et al., 2001b, and in this study $r = -0.12$). Indeed, the largest change in BP during sleep appears to be during the sleep onset process (Trinder et al., 2001). Nonetheless, as with HF and respiratory parameters, a cautious approach when recording PEP would be to also record BP.

In Table 4 we have highlighted the apparent advantages and disadvantages of HF, LF, PEP and rRR. HF is clearly the best noninvasive estimate of cardiac parasympathetic activity. While PEP is the only noninvasive measure of cardiac sympathetic activity that has withstood thorough pharmacological testing (Cacioppo et al., 1994; Schachinger et al., 2001), it only changes slightly from NREM to REM sleep. Other measures, such as microneurographic recordings (e.g. Somers et al., 1993) and plasma norepinephrine concentrations (e.g. Dodt et al., 1997) offer more direct assessment of sympathetic activity, but are more likely to disturb sleep. Furthermore, these latter measures do not specifically estimate cardiac sympathetic activity, which due to fractionation in the autonomic system may differ from sympathetic outflow to other peripheral systems (Berntson et al., 1997; Schachinger et al., 2001). Thus the method chosen to estimate sympathetic activity in humans is probably best decided upon once the design of a study is determined.

Our results indicate that rRR is capable of tracking sympathovagal changes that occur between NREM and

Table 4
The advantages and disadvantages of the measures of cardiac autonomic activity

	Advantages	Disadvantages
HF	Measure of parasympathetic activity	Confounded by variations in respiratory activity
	Inexpensive	
	Fast expert analysis	
LF	Inexpensive	Mixed measure of parasympathetic and sympathetic activity
	Fast expert analysis	
PEP	Measure of sympathetic activity	Requires an impedance cardiograph
		Time consuming expert analysis
		Confounded by variations in blood pressure
		Small changes between NREM and REM sleep
rRR	Inexpensive	Mixed measure of parasympathetic and sympathetic activity
	Fast simple analysis	Not yet thoroughly pharmacologically validated

REM sleep, but as a single measure it cannot differentiate between an increase in sympathetic and decrease in parasympathetic activity or vice versa. Its ease of calculation and large changes during sleep suggest that following its pharmacological validation, it may prove a useful measure to calculate when exploring data for potential sympathovagal changes.

Acknowledgements

We thank Drs. Kathyrn Reid and John Trinder for their comments on the manuscript. This work was supported by the following grants: NIH grants DK-41814 and AG-11412 and The University of Chicago Clinical Research Center grant RR00055.

References

Akselrod S, Gordon D, Ubel FA, Shannon DC, Barger AC, Cohen RJ. Power spectrum analysis of heart rate fluctuation: A quantitative probe of beat-to-beat cardiovascular control. Science 1981;213:220–2.

Ako M, Kawara T, Uchida S, Miyazaki S, Nishihara K, Mukai J, Hirao K, Ako J, Okubo Y. Correlation between electroencephalography and heart rate variability during sleep. Psychiatry Clin Neurosci 2003;57:59–65.

Baharav A, Kotagal S, Gibbons V, Rubin BK, Pratt G, Karin J, Akselrod S. Fluctuations in autonomic nervous activity during sleep displayed by power spectrum analysis of heart rate variability. Neurology 1995;45: 1183–7.

Berntson GG, Cacioppo JT, Quigley KS. Respiratory sinus arrhythmia: autonomic origins, physiological mechanisms, and psychophysiological implications. Psychophysiology 1993;30:183–96.

Berntson GG, Cacioppo JT, Binkley PF, Uchino BN, Quigley KS, Fieldstone A. Autonomic cardiac control. III. Psychological stress and cardiac response in autonomic space as revealed by pharmacological blockades. Psychophysiology 1994;31:599–608.

Berntson GG, Bigger JT, Eckberg DL, Grossman P, Kaufmann PG, Malik M, Nagaraja HN, Porges SW, Saul JP, Stone PH, van der Molen MW. Heart rate variability: origins, methods, and interpretive caveats. Psychophysiology 1997;34:623–48.

Bonnet MH, Arand DL. Heart rate variability: sleep stage, time of night, and arousal influences. Electroenceph clin Neurophysiol 1997;102: 390–6.

Brandenberger G, Ehrhart J, Piquard F, Simon C. Inverse coupling between ultradian oscillations in delta wave activity and heart rate variability during sleep. Clin Neurophysiol 2001;112:992–6.

Burgess HJ, Trinder J, Kim Y. Cardiac parasympathetic nervous system activity does not increase in anticipation of sleep. J Sleep Res 1996;5: 83–9.

Burgess HJ, Trinder J, Kim Y, Luke D. Sleep and circadian influences on cardiac autonomic nervous system activity. Am J Physiol 1997;273: H1761–8.

Burgess HJ, Trinder J, Kim Y. Cardiac autonomic nervous system activity during presleep wakefulness and Stage 2 NREM sleep. J Sleep Res 1999;8:113–22.

Burgess HJ, Holmes AL, Dawson D. The relationship between slow-wave activity, body temperature, and cardiac activity during nighttime sleep. Sleep 2001a;24:343–9.

Burgess HJ, Sletten T, Savic N, Gilbert SS, Dawson D. Effects of bright light and melatonin on sleep propensity, temperature, and cardiac activity at night. J Appl Physiol 2001b;91:1214–22.

Cacioppo JT, Berntson GG, Binkley PF, Quigley KS, Uchino BN, Fieldstone A. Autonomic cardiac control. II. Noninvasive indices and basal response as revealed by autonomic blockades. Psychophysiology 1994;31:586–98.

Cajochen C, Pischke J, Aeschbach D, Borbely AA. Heart rate dynamics during human sleep. Physiol Behav 1994;4:769–74.

de Geus EJC, Willemsen GHM, Klaver CHAM, van Doornen LJP. Ambulatory measurement of respiratory sinus arrhythmia and respiration rate. Biol Psychol 1995;41:205–27.

Dodt C, Breckling U, Derad I, Fehm HL, Born J. Plasma epinephrine and norepinephrine concentrations of healthy humans associated with nighttime sleep and morning arousal. Hypertension 1997;30:71–6.

Ehrhart J, Toussaint M, Simon C, Gronfier C, Luthringer R, Brandenberger G. Alpha activity and cardiac correlates: three types of relationships during nocturnal sleep. Clin Neurophysiol 2000;111:940–6.

Elsenbruch S, Harnish MJ, Orr WC. Heart rate variability during waking and sleep in healthy males and females. Sleep 1999;22:1067–71.

Ferini-Strambi L, Spera A, Oldani A, Zucconi M, Bianchi A, Cerutti S, Smirne S. Autonomic function in narcolepsy: power spectrum analysis of heart rate variability. J Neurol 1997;244:252–5.

Harris AS, Burgess HJ, Dawson D. The effects of day-time exogenous melatonin administration on cardiac autonomic activity. J Pineal Res 2001;31:199–205.

Kamath MV, Fallen EL. Power spectral analysis of heart rate variability: A noninvasive signature of cardiac autonomic function. Crit Rev Biomed Eng 1993;21:245–311.

Kamen PW, Krum H, Tonkin AM. Poincare plot of heart rate variability allows quantitative display of parasympathetic nervous activity in humans. Clin Sci 1996;91:201–8.

Malliani A, Pagani M, Lombardi F, Cerutti S. Cardiovascular neural regulation explored in the frequency domain. Circulation 1991;84: 482–92.

Malliani A, Lombardi F, Pagani M. Power spectrum analysis of heart rate variability: a tool to explore neural regulatory mechanisms. Br Heart J 1994;71:1–2.

Mancia G, Zanchetti A. Cardiovascular regulation during sleep. In: Orem J, Barnes CD, editors. Physiology in sleep. New York: Academic Press; 1980. p. 1–55.

Ori Z, Monir G, Weiss J, Sayhouni X, Singer DH. Heart rate variability. Frequency domain analysis. Cardiol Clin 1992;10:499–537.

Otzenberger H, Simon C, Gronfier C, Brandenberger G. Temporal relationship between dynamic heart rate variability and electroencephalographic activity during sleep in man. Neurosci Lett 1997;229:173–6.

Otzenberger H, Gronfier C, Simon C, Charloux A, Ehrhart J, Piquard F, Brandenberger G. Dynamic heart rate variability: a tool for exploring sympathovagal balance continuously during sleep in men. Am J Physiol 1998;275:H946–50.

Pomeranz B, Macaulay RJB, Caudill MA, Kutz I, Adam D, Gordon D, Kilborn KM, Barger AC, Shannon DC, Cohen RJ, Benson H. Assessment of autonomic function in humans by heart rate spectral analysis. Am J Physiol 1985;248:H151–3.

Rechtschaffen A, Kales A. A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Washington, DC: Public Health Service/US Government Printing Office; 1968.

Schachinger H, Weinbacher M, Kiss A, Ritz R, Langewitz W. Cardiovascular indices of peripheral and central sympathetic activation. Psychosom Med 2001;63:788–96.

Schechtman VL, Raetz SL, Harper RK, Garfinkel A, Wilson AJ, Southall DP, Harper RM. Dynamic analysis of cardiac R-R intervals in normal infants and in infants who subsequently succumbed to the sudden infant death syndrome. Pediatr Res 1992;31:606–12.

Sherwood A, Allen MT, Fahrenberg J, Kelsey RM, Lovallo WR, van Doornen LJP. Methodological guidelines for impedance cardiography. Psychophysiology 1990;27:1–23.

Somers VK, Dyken ME, Mark AL, Abboud FM. Sympathetic-nerve activity during sleep in normal subjects. N Engl J Med 1993;328:303–7.

Spiegel K, Leproult R, Van Cauter E. Impact of sleep debt on metabolic and endocrine function. Lancet 1999;354:1435–9.

Thakor NV, Webster JG, Tompkins WJ. Optimal QRS detector. Med Biol Eng Comput 1983;21:343–50.

Trinder J, Kleiman J, Carrington M, Smith S, Breen S, Tan N, Kim Y. Autonomic activity during human sleep as a function of time and sleep stage. J Sleep Res 2001;10:253–64.

Umali MU, Hilton MF, Kres SP, Czeisler CA, Wyatt JK, Shea SA. Circadian and sleep stage influences on cardiac autonomic tone. Sleep 2000;23:A26–7.

Van De Borne P, Nguyen H, Biston P, Linkowski P, Degaute JP. Effects of wake and sleep stages on the 24-h autonomic control of blood pressure and heart rate in recumbent men. Am J Physiol 1994;266:H548–54.

Willemsen GHM, de Geus EJC, Klaver CHAM, van Doornen LJP, Carroll D. Ambulatory monitoring of the impedance cardiogram. Psychophysiology 1996;33:184–93.

Zemaityte D, Varoneckas G, Sokolov E. Heart rhythm control during sleep. Psychophysiology 1984;21:279–89.