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ORIGINAL ARTICLE

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Circadian variation in base rate measures of cardiac autonomic activity

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Abstract To investigate the role of the circadian pacemaker in autonomic modulation of base rate cardiac activity, 29 healthy subjects participated in a constant routine protocol. They were randomly divided into two groups in order to manipulate prior wakefulness. Group 1 started at 0900 hours immediately after a monitored sleep period, while group 2 started 12 h later. Measures of interbeat intervals (IBIs), respiratory sinus arrythmia (RSA, an estimate of parasympathetic activity), pre-ejection period (PEP, an estimate of sympathetic activity), and core body temperature (CBT) were recorded continuously. Multilevel regression analyses (across-subjects) revealed significant 24- and/or 12-h sinusoidal circadian variation for CBT, IBI, and RSA, but not for PEP. Subject-specific 24 + 12 h sinusoidal fits demonstrated a convergence of phase distribution for IBI and RSA of group 1 similar to CBT, while PEP showed a relatively large (i.e. random) distribution of phase. In group 2, all cardiac measures showed large distributions of phase. Unexpected results in the cardiac measures were found in group 2, probably caused by group differences in prior activation. Also, effects of sleep deprivation were observed for IBI and RSA in group 2. Consequently, all cardiac measures revealed significant sinusoidal × group interactions, a result not shown in CBT. These findings were interpreted as an indication for circadian endogenous parasympathetic modulation of cardiac activity that is mainly confounded by prior wakefulness that extends 24 h, while the sympathetic modulation is relatively uncoupled from the endogenous circadian drive and mainly influenced by prior activation.

Keywords Circadian variation · Constant routine · Core body temperature · Parasympathetic activity · Sympathetic activity

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Introduction

Circadian rhythmicity—fluctuations with an intrinsic period of about 24 h—exists in psychological as well as in physiological functions (Moore-Ede et al. 1982). Cognitive performance (Monk et al. 1997), activity of the hypothalamic–pituitary–adrenocortical axis (Buijs et al. 1999), heart rate (Kräuchi and Wirz-Justice 1994), and core body temperature (CBT; Watts 1991) represent parameters that are strongly modulated by an endogenous clock mechanism.

Neuroanatomical studies have revealed the central physiological mechanisms most likely responsible for endogenous circadian rhythmicity in these parameters. Projections have been demonstrated from the suprachiasmatic nucleï (SCN, the so-called biological clock) to hypothalamic and prefrontal areas that are involved in hormone secretion, cardiovascular regulation, and (cognitive) behavioral activity (Dai et al. 1998). Additionally, projections have been found from the SCN to the locus coeruleus and the adrenal cortex (Buijs et al. 1998, 1999; Dai et al. 1998; Aston-Jones et al. 2001). Evidence of cardiac modulation by the SCN via (multisynaptic) autonomic pathways has been established in mechanistic animal models (Warren et al. 1994; Scheer et al. 2001) and was also strongly suggested in humans (Scheer et al. 1999). Thus, central autonomic regulatory mechanisms are likely to be modulated by the output of the SCN, indicating that the SCN is able to transmit its time-of-day signal to the heart and other target organs. However, the relative contribution of the sympathetic

and parasympathetic branches in this modulation is still unclear.

The central objective of the current study was to investigate endogenous sympathetic and/or parasympathetic circadian modulation of base rate cardiac autonomic activity. This variation could be an important underlying mechanism for the time-dependent distributions of cardiac events such as angina pectoris attacks (Nademanee et al. 1987) and transient myocardial ischemia (Rocco et al. 1987).

It has now been established that endogenous circadian rhythmicity can only be assessed under strictly controlled conditions such as defined by the constant routine protocol. This protocol was originally proposed by Mills and associates (Mills et al. 1978) and was later modified by Czeisler and colleagues (1985). In the constant routine protocol, the evoked effects of change in exogenous environmental and behavioral conditions are eliminated or evenly distributed across the 24-h period, which enhances the reliability of assessment of the core circadian pacemaker characteristics and its direct contribution to rhythmicity in different variables (Kerkhof and van Dongen 1996; Kerkhof et al. 1998; Duffy and Dijk 2002). Only a small number of studies have employed a constant routine protocol and have found 24-h patterns in heart rate (Kräuchi and Wirz-Justice 1994; van Dongen et al. 2001) and parasympathetic activity (Burgess et al. 1997). However, 24-h patterns in sympathetic activity have not been demonstrated (Burgess et al. 1997). The current study made use of a constant routine protocol in order to effectively study base rate measures of cardiac autonomic activity (sympathetic and parasympathetic) as a function of the circadian pacemaker.

A confounder of the constant routine protocol is the accumulation of sleep deprivation and the associated changes in physiological and subjective variables (e.g. heart rate, body temperature, sleepiness and fatigue; see Holmes et al. 2002; van Dongen et al. 2003). Therefore, not one but two regulatory processes are involved: a circadian and a homeostatic process (Borbély 1982; see also van de Borne et al. 1994). The current study, therefore, also attempted to estimate the presence of sleep deprivation effects by randomly dividing the subjects into an early-start (0900 hours) group and a late-start (2100 hours) group. In this way, the potential contributions of endogenous circadian rhythmicity and sleep deprivation to base rate cardiac autonomic activity could be estimated.

Methods

Subjects

The subjects, 33 Dutch students of the University of Leiden [15 males, 18 females; mean (SD) age, 21.8 (4.1) years; body-mass index, 21.7 (2.0) ko/M^2] were recruited through advertising and were paid for their contribution to the experiment. Ambulatory sleep/wake cycles were assessed 2 weeks prior to the experiment by means of a logbook. Mean bed-in and bed-out times were 0039 hours (58 min SD) and 0917 hours (62 min SD) respectively. Of the 18 females, 7 were in the follicular phase and 11 in the luteal phase of their menstrual cycle; 10 female subjects used oral contraceptives.

Subjects assigned to the early-start group and the late-start group were comparable in age, sex, body-mass index, mean bed-in and bed-out times, menstrual phase, and anti-conception use. Medical and psychological screening was carried out to ensure that the subjects were healthy, free from medication, and had no personal or family history of clinical sleep disorders or major psychopathology. Subjects reported not to have had any shift work or transmeridian travel in the past 3 months, or any accidents or surgery in that period of time. They were not experiencing any major life stress and had no examinations scheduled for a few days before, during, or after the study.

Excessive smokers (≥ 5 cigarettes), caffeine users (≥ 350 MG/DAY), alcohol users (≥ 5 standard drinks per week) and physical exercisers (>4 h per week) were excluded from the study. The experimental protocol and subjects' rights comply with the current Dutch laws.

Experimental design

To assess the relative contribution of the endogenous circadian system to changes in cardiac activity, a constant routine procedure was used. This procedure aims to control for the masking effects of light and food intake. Also, sleep, postural, and physical activity changes were accounted for by requiring subjects to remain awake in a semi-recumbent position for a period of >24 h. The environment in which the subjects resided during the constant routine was closed off from the external world, and lighting conditions (30–50 lx, as measured in the horizontal angle of gaze) and temperature conditions (18°C) were kept constant. The effect of food intake was also evenly distributed by providing small meals (100–120 kcal) every hour. Subjects were under constant surveillance of the experimenters to assure that they were awake. The subject pool was randomly split into two groups. The first group contained 18 subjects who started the constant routine protocol at 0900 hours until 1200 hours the next day. The second group contained 15 subjects who started 12 h later at 2100 hours until 0000 hours the next day.

Procedure

The study was conducted in the sleep laboratory at the Faculty of Social Science, University of Leiden, The Netherlands. Each subject was part of a multifaceted study in circadian rhythmicity of base rate physiological

and psychological measures as well as physiological responses to mental and physical challenges. The data presented here comprise a segment of this set.

After the screening and consent procedures, all subjects came in the laboratory facility the first evening at 2200 hours. Subjects then slept in the sleep laboratory to get adapted to the experimental environment. The next morning, they were allowed to spend the day freely, with the exception of naps. The second evening, all subjects returned to the laboratory at 2200 hours for a sleep registration night. Polysomnographic, cardiac, and CBT measurements were collected during the sleep period. The habitual bedtime and waketime of each subject was taken into account within the range of 2330–0030 hours (lights off) and 0730–0830 hours (lights on). At 0900 h, subjects of group 1 assumed a semi-recumbent position on a bed (a metal rack supported the mattress to an angle of 45 deg as back support) to which they were confined for the next 27 h, with the exception of toilet requirements. The first 2 h of the constant routine procedure were considered adaptation hours. The subjects remained connected to the cardiac and temperature devices at all time.

After waking up between 0730 and 0830 hours, subjects of group 2 were allowed to spend the day freely, with the exception of naps. In the evening, these subjects returned to the laboratory at 2000 hours, and were again connected to the cardiac and the temperature devices. At 2100 hours, they also began their constant routine procedure. As part of another study, a task battery composed of four cognitive computer tasks and, lastly, a cold pressor test, was presented at 1100 hours (or at 2300 hours in group 2) and was repeated every 3 h throughout the experiment. These data are not reported here.

Every hour, subjects were allowed to choose one snack of 100–120 kcal from a list of 20, accompanied by 150 ml of water or sugar- and caffeine-free soda. Subjects were encouraged to eat every hour, but it was not mandatory to do so. Additional access to water was granted except during or just prior to data collection. The subjects were permitted to listen to music, read, and have casual conversations with the experimenters and to watch non-arousing videos during the experiment. All subjects were aware of clock time and allowed to wear watches.

Assessment of physiological measurements

Recordings of heart activity were made continuously with the Vrije Universiteit Ambulatory Monitoring System (VU-AMS; version 4.6, TD-FPP). This device uses six Ag/AgCl electrodes to record interbeat intervals and thoracic impedance (d Z), and also gives an indication of the amount of motor activity (motility). Details on electrode placement and R-spike detection of this device can be found in de Geus et al. 1995. Cross-instrumental comparison of the VU-AMS with a standard laboratory measurement set-up showed excellent for between-subject and within-subject correlations of heart rate, respiratory rate, respiratory sinus arrythmia, and spectral heart period powers (de Geus et al. 1995).

Heart rate was measured in the current study as interbeat intervals (IBIs): R-R intervals derived from the ECG, expressed in milliseconds. Parasympathetic cardiac (vagal) activity was measured as rhythmic variations in heart rate at the frequency of respiration, defined as respiratory sinus arrythmia (RSA). RSA is generally believed to be mediated predominately by fluctuations of vagal–cardiac nerve traffic and thus may provide an index of vagal activity (Berntson et al. 1997). A number of studies showed that RSA can be estimated as the heart period power value within the respiratory high-frequency (HF) interval (0.125–0.50 Hz) (Berntson et al. 1997).

Sympathetic cardiac activity was measured as the isovolumetric contraction time of the ventricle, defined as pre-ejection period (PEP). PEP is obtained by means of alterations in thoracic impedance, and is inversely related to myocardiac contractility. Also, it is frequently used in psychophysiological research as a non-invasive measure of sympathetic modulation of heart activity (Sherwood et al. 1990; van Doornen and de Geus 1996). Moreover, it especially proved to be a good measure for sympathetic modulation when subjects are in a resting position (Cacioppo et al. 1994).

Base rate CBT was used in the current study as a biological marker of SCN activity. CBT was measured every 6 min with a rectal thermistor (Yellow Springs Instruments) and recorded on a Smart Reader (ASKEY).

Data analyses

Data reduction

Data periods of 10 min in length, collected just prior to the onset of a task battery (i.e. once every 3 h), were taken from the cardiac dataset for the current study. The heart period (IBI) data of each participant were analyzed in segments representing 32 s. An artifact preprocessing was performed on these segments by detecting outlier IBI values with two methods: (a) by absolute values (>1800 ms or <300 ms) and (b) a moving average filter (>3 SD deviation from the moving mean). Since artifacts cannot simply be deleted because the continuity of time would be lost, spuriously short IBIs were summed and missing beats were interpolated by splitting spuriously long IBIs. The IBI mean values were computed from these corrected data.

Next, all segments (32 s) were subjected to a discrete Fourier transformation. Heart period power values were computed for the HF band (0.125–0.50 Hz). The power values were $\log_{10}$ transformed to obtain normal distributions.

The dZ/dt values [obtained from the thoracic impedance (dZ) data sampled at 250 Hz around each R-wave] were ensemble averaged over the corresponding segments. The B-points (onset of dZ/dt upstroke) were manually determined for each ensemble-averaged segment, and the PEP values were determined by summing a fixed Q-wave-to-R-wave interval of 48 ms to the R-B interval time (de Geus et al. 1995). Finally, the mean IBI, RSA, and PEP values were computed for each data period to obtain reliable time series estimates of base rate cardiac activity.

Outliers in the CBT data (defined as acute temperature changes that exceeded 0.25°C) were marked as missing values. Three subjects (two from the early-start group and one from the late-start group) were excluded from further temperature analysis because of equipment failure. Missing values in the remaining subjects that resulted from probe-related errors were linearly interpolated. A selection of the temperature data set, one sample every 3 h similar to the cardiac data, was used for the multilevel analysis (see below).

Multilevel (across subjects) regression analysis

It is known from previous studies using CBT (see Brown and Czeisler 1991) that this biological marker of SCN activity has a circadian as well as an ultradian component, and harmonizes with a 24 + 12 h cosine fit. To test for the contributions of the 12 h and 24 h sinusoidal fluctuations across subjects, a regression analysis was employed on the cardiac and temperature data. A complication in the regression design, as used in the current study, is that observations within each subject are correlated (i.e. are not independent). However, multilevel regression analysis (MLwiN; version 1.100006) allows differentiation between two types of variance, between-individual variance and within-individual variance, taking into account the dependencies that are present in the data and adjusts the standard errors of the estimated regression coefficients accordingly (Rasbash et al. 2000; Hox 2002). It is also robust against violations against homoschedasticity and sphericity, which leads to a more conservative estimation of main effects.

The basis of the 24 + 12 h regression model:

$$f(t) = a_1\sin[2\pi(t/24) + b_1] + a_2\sin[2\pi(t/12) + b_2] + c$$

can be rewritten using the formula:

$$\sin(a + b) = \sin(a)\cos(b) + \cos(a)\sin(b) \text{ to:}$$

$$f(t) = X1\sin(2\pi t/24) + X2\cos(2\pi t/24) + X3\ \sin(2pt/12) + X4\cos(2\pi t/12) + c$$

with $X1 = a_1\cos(b_1)$, $X2 = a_1\sin(b_1)$, $X3 = a_2\cos(b_2)$, and $X4 = a_2\sin(b_2)$

Hence, four predictors related to endogenous circadian factors are present in the model: $\sin(2\pi t/24)$, $\cos(2\pi t/24)$, $\sin(2\pi t/12)$, and $\cos(2\pi t/12)$. Thus, the multilevel regression formula, with the two levels subjects and time number, is:

$$\begin{aligned} f(t) = {} & b_1\cos[2\pi(t/24)] + b_2\sin[2\pi(t/24)] \\ & + b_3\cos[2\pi(t/2)] + b_4\sin[2\pi(t/12)] + b_5\text{group} \\ & + b_6\text{group}\cos[2\pi(t/24)] + b_7\text{group}\sin[2\pi(t/24)] \\ & + b_8\text{group}\cos[2\pi(t/12)] + b_9\text{group}\sin[2\pi(t/12)] + c \end{aligned}$$

Four β-values (fixed factors) were obtained that represent the (across subjects) 12 h and 24 h sinusoidal main effects (i.e. b_1–b_4 representing the amplitudes and phases). Additionally, the main effect of group is modeled by a dummy variable (value 0 or 1), and four additional terms (b_6–b_9) were modeled representing the group × sinusoidal interactions. The relatively low number of subjects did not permit exploration of random factors. To facilitate interpretation, standardized β-values were calculated (i.e. standardized to values between 0 and 1).

Individual fit procedure

Individual differences in sinusoidal phases were explored by fitting individual data profiles with a combined 24 h fundamental and a 12 h harmonic sinusoidal curve. Analysis were carried out with custom made software, using the formula:

$$f(t) = a_1\sin[2\pi(t/24 + b_1)] + a_2\sin[2\pi(t/12 + b_2)] + c$$

with t = sample time, a_1 and a_2 amplitude parameters, b_1 and b_2 phase parameters (ranging from 0 to 1), and c an offset ($c \approx 0$). Since two frequencies were combined, the phase estimation was determined by using the absolute minimum (in CBT) and maximum (in the cardiac measures) of the interpolated curve.

Group differences in the individual fitted phase estimations were tested non-parametric using the Mann–Whitney U test. Within-group correlations were tested non-parametric using Spearman's rho.

Results

Mean (across subject) CBT

The mean CBT values are shown in Fig. 1. A distinct and 24 h sinusoidal pattern is visible for both groups, with maximal values around 2000 hours and minimal values around 0500 hours. The standardized β-values obtained from the multilevel regression analysis are presented in Table 1. As expected, a significant and large 24 h main sinusoidal effect was found for CBT. The fact that only the sinus component (and not the cosinus) was found indicates that the phase was completely in harmony with a sinus, as also can be observed in Fig. 1. No main group effects or sinusoidal × group interactions were found for CBT.

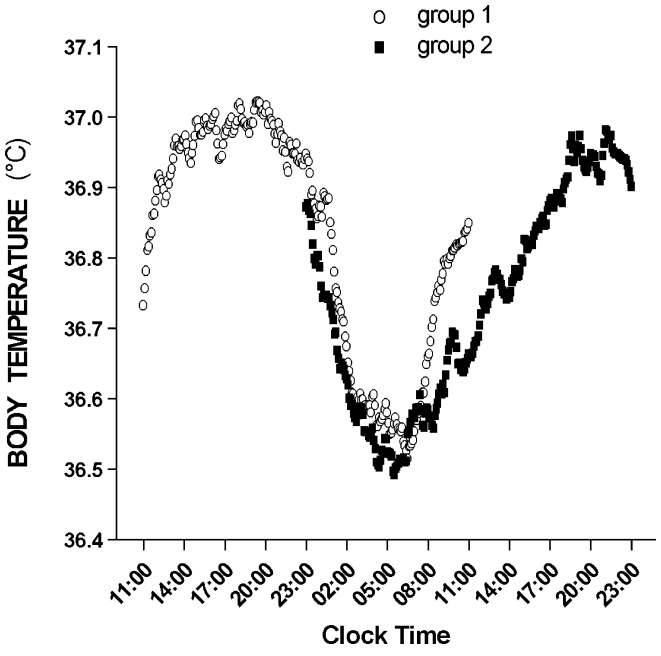

Fig. 1 Core body temperature was measured every 6 min for a period of 24 h. All samples are plotted as a function of clock time

Table 1 The contributions of the 12-h and 24-h sinusoidal functions in the raw cardiac measures and core body temperature (*CBT*) were derived from multilevel analysis and are expressed as standardized β-values. *IBI* Interbeat interval, *RSA* respiratory sinus arrythmia, *PEP* pre-ejection period

	IBI	RSA	PEP	CBT
Sinusoidal effects				
Cosinus 24-h	0.09	0.04	0.02	0.04
Sinus 24-h	0.17**	0.13*	0.04	0.45***
Cosinus 12-h	−0.03	−0.03	0.02	0.08
Sinus 12-h	0.13*	0.13*	0.01	−0.02
Group effects	0.26	0.24	0.20	−0.13
Sinusoidal × group effects				
Cosinus 24-h	−0.15*	−0.13*	−0.04	0.07
Sinus 24-h	−0.15*	−0.09	−015**	0.05
Cosinus 12-h	−0.04	−0.02	−0.03	−0.03
Sinus 12-h	−0.11*	−0.13*	−0.02	−0.05

Significance of variance: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$

Mean (across subjects) cardiac activity

Interbeat interval

The mean IBI values across the subjects are shown in Fig. 2. Group 1 showed a sinusoidal pattern: relative low values during the diurnal hours, except for a minor elevation at 1400 hours. A minimum was reached at 2000 hours, followed by a steady increase during the night towards a maximum at 0500 hours. After that, a sharp decrease was observed in the morning hours towards about the same value as the initial sample. Group 2 showed a pattern congruent with that of group 1 during the night until 0500 hours, but failed to show the subsequent decrease as observed in group 1. Instead, group 2 continued with a modest linear increase during the diurnal hours. The multilevel regression analysis revealed significant main sinusoidal effects for the 24 h and for the 12 h sinus function for IBI. Note that despite the significance of these effects, the corresponding standardized β-values remained relatively small. No main effect of group was found. However, the differences between the two groups in the chronological course of the cardiac data, as seen in Fig. 2, was reflected in significant sinusoidal × group interactions.

Respiratory sinus arrythmia

The mean RSA values across the subjects are shown in Fig. 3. Group 1 showed a sinusoidal pattern: relative low values during the diurnal hours, except for a minor elevation at 1400 hours and 1700 hours. A minimum was reached at 2300 hours, followed by a steady increase during the night towards a maximum at 0200 hours and 0500 hours. After that, a subsequent decrease was observed in the morning hours. The first sample (i.e. at 2300 hours) of group 2 was elevated in comparison to group 1, after which RSA showed a steady increase towards a maximum value at 0800 hours. RSA values then slightly decreased during the remainder of the experiment. The multilevel regression analysis revealed significant main Sinusoidal effects (for the 24 h and for the 12 h sinus) for RSA. Again, relatively small, standardized β-values were observed, a main effect of group was absent, and significant sinusoidal × group interactions were found.

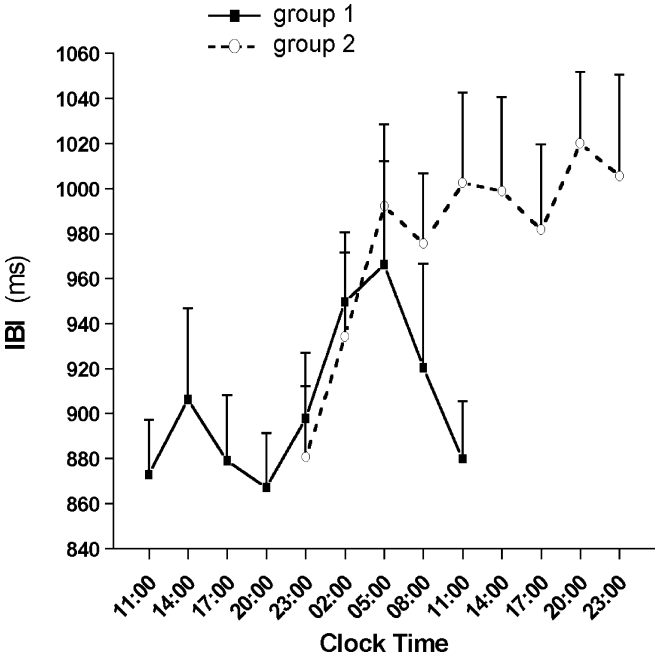

Fig. 2 Raw values of the interbeat intervals (*IBI*, in milliseconds) for group 1 and group 2 are plotted as a function of clock time. Bars represent SEM

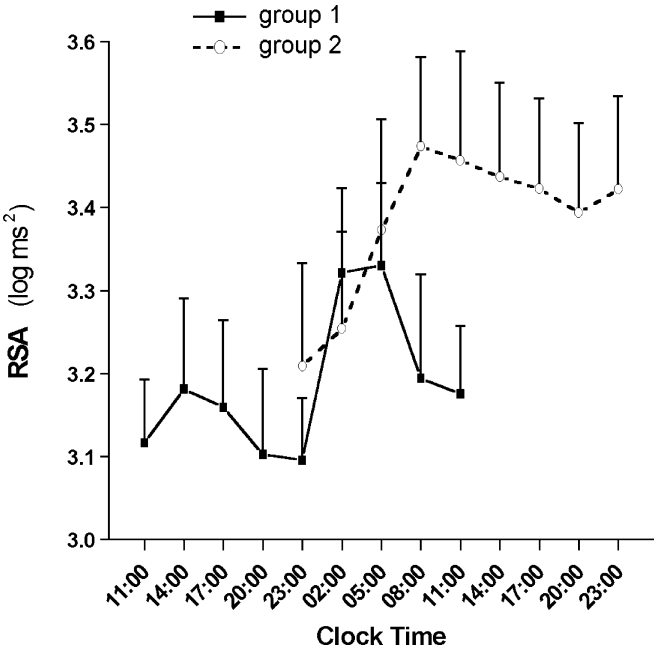

Fig. 3 Raw values of the respiratory sinus arrythmia (*RSA*, in log transformed power) for group 1 and group 2 are plotted as a function of clock time. Bars represent SEM

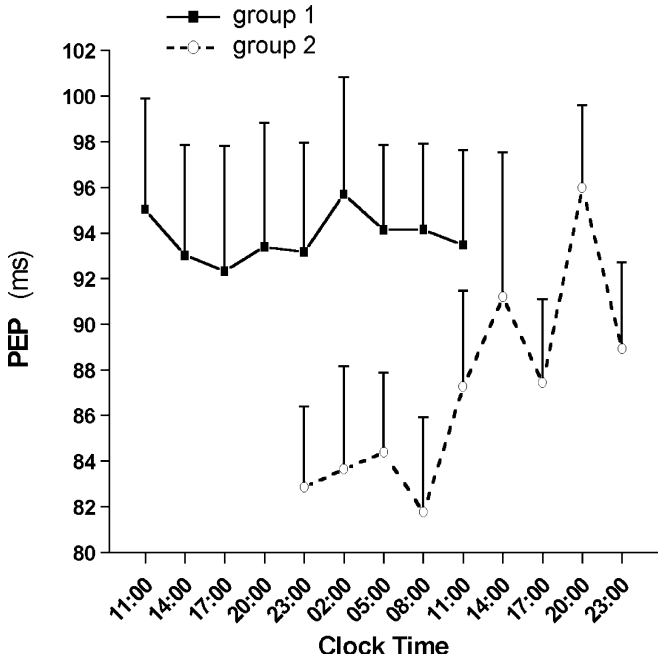

Fig. 4 Raw values of the pre-ejection period (*PEP*, in milliseconds) for group 1 and group 2 are plotted as a function of clock time. Bars represent SEM

Pre-ejection period

The mean PEP values across the subjects are shown in Fig. 4. Group 1 showed an unclear pattern: regardless of time of day, the mean values remained fairly stable around 94 ms. Group 2, however, showed low values during the night, with a minimum value at 0800 hours. After that, a sharp increase was observed towards a maximum at 2000 hours, followed by a decrease towards the end of the experiment at 2300 hours. In contrast to IBI and RSA, the multilevel regression analysis did not show significance main sinusoidal effects for PEP. A main effect of group was also absent. However, a significant sinusoidal × group interaction was found for PEP.

Individual fit procedure

The individual phase estimations are presented in Table 2. Significant group differences were found in the mean phase estimations for IBI ($Z = -3.723$, $P < 0.001$), RSA ($Z = -4.343$, $P < 0.001$), and PEP ($Z = -4.115$, $P < 0.001$), but not for CBT ($Z = -0.128$, $P = 0.898$). Interestingly, for group 1, IBI showed a mean maximum value that was phase-correlated to that of RSA ($r = 0.626$, $P = 0.017$), but not to that of PEP. However, within group 2 none of the cardiac measures were phase related.

These group differences in phase estimations are clarified by observing the across subject variations (SDs of phase distribution). A marked similarity was found for group 1 between the relatively small standard deviations of IBI and RSA similar to core body temperature, as shown in Table 2, while this value was much larger for PEP. However, for group 2 the SDs were relatively large for IBI, RSA, and PEP, while this value was only relatively small for CBT. In both groups, no correlations were observed between cardiac measures and CBT.

Discussion

The current study, performed to demonstrate endogenous circadian rhythmicity and the effects of sleep deprivation in base rate cardiac activity, obtained three primary results. First, based on the chronological plotted mean cardiac data (i.e. Figs. 2, 3, 4) and the results from the multilevel analyses, heart rate, and cardiac vagal activity showed a characteristic circadian rhythmicity when monitored during the initial 24 h of the constant routine (group 1). Sympathetic activity, however, failed to mirror these results. Second, all cardiac measures showed sinusoidal × group interactions. When prior wakefulness was extended by 12 h (group 2), marked differences in the course of IBI, RSA, and PEP were observed. Third, based on the individual sinusoidal fits, large between-subject distributions of phase estimations were observed, particularly in group 2.

Only a few studies thus far have examined the two subsystems of the autonomic nervous system using a constant routine protocol (Burgess et al. 1997; Kräuchi et al. 2000; Holmes et al. 2002), and they are in general agreement with the results presented in this paper. The first study, conducted by Burgess et al. (1997), demonstrated a distinct 24 h sinusoidal pattern in heart rate

Table 2 The absolute minimum (*Phase min*) in CBT and absolute maximum (*Phase max*) in the cardiac measures were taken from the individual fit profiles to observe the distribution of phase estimation for group 1 and group 2. *NS* Non-significant fit, *ND* missing data due to equipment failure

Subjects	IBI[b] Phase max (h)	RSA[b] Phase max (h)	PEP[b] Phase max (h)	CBT Phase min(h)
Group 1				
1	3:05	0:37	11:53	ND
2	5:21	4:29	4:37	5:05
3	23:35	23:02	13:29	1:22
4	6:03	6:16	13:34	2:06
5	1:17	3:26	12:08	6:47
6	1:01	1:07	9:40	4:18
7	3:17	3:21	8:11	2:23
8	2:00	5:18	4:07	6:31
9	3:25	5:11	5:23	5:10
10	1:20	2:01	1:35	5:43
11	NS	4:10	18:13	7:47
12	4:55	5:10	14:50	6:16
13	2:50	23:39	NS	ND
14	5:13	4:25	6:13	6:11
15	2:49	2:55	19:30	5:48
Mean (SD)[a]	3:01(1:53)	3:08(2:12)	22:15(8:11)	5:02(1:57)
Group 2				
1	8:40	8:19	16:41	ND
2	19:12	18:44	19:48	4:48
3	NS	6:09	18:02	4:52
4	15:36	8:19	19:14	9:03
5	22:12	10:54	18:49	3:20
6	NS	NS	14:29	3:22
7	NS	6:59	NS	4:45
8	18:07	8:54	18:15	2:46
9	18:51	23:23	10:37	3:38
10	10:03	7:05	NS	7:51
11	17:25	22:23	21:36	4:11
12	12:14	12:30	12:20	8:13
13	7:36	12:12	15:10	8:48
14	2:30	NS	4:53	3:35
Mean (SD)[a]	13:51(6:05)	12:29(5:52)	15:49(4:42)	5:19(2:17)

[a]Mean (SD) of significant fits
[b]The level of significance for these measurements was set to $P < 0.1$ because of the limited number of data points

and parasympathetic activity, whereas sympathetic activity remained markedly stable under circumstances of continuous sleep deprivation (see also Kräuchi et al. 2000). These patterns of results are analogous to our findings, except for RSA that increased prior to the supposed sleep period (i.e. started to increase at 2000 hours) and peaked at about 0100 hours. With reference to this result, the pattern of RSA observed in our study shows a delay of about 3 h. Also, comparable to our multilevel analysis, the average cardiac data was modeled to a 24 + 12 h sinusoidal fit, and found that PEP was not fit by a simple (24 h) or a complex (24 + 12 h) curve, heart rate was best fit by a simple (24 h) curve, and RSA was best fit by a complex (24 + 12 h) curve. Furthermore, Burgess et al. (1997) assigned half of the subjects to an early-start group (i.e. data collection began at 1200 hours) and the other half to a late-start group (i.e. data collection began at 2100 hours), so as not to confound the circadian phase and sleep with time in the experiment. The differences found resulted from the late-start group not showing a main effect of time-of-day for PEP, whereas in the early-start group this did reach significance. In contrast, heart rate had a significant main effect of time-of-day in the early-start group, but not in the late-start group.

Another study that employed a constant routine circadian protocol was performed by Holmes et al. (2002). They required subjects to participate in two experimental conditions, i.e. high sleep pressure (30 h of wakefulness) and low sleep pressure (6 h of wakefulness). Their results indicated that the accumulation of prior wakefulness down-regulates the activity of the heart, with decreased sympathetic activity underlying this effect. The results of the current study also clearly showed down-regulation of heart rate (i.e. increased IBI), but with both increased parasympathetic activity (increased RSA) and decreased sympathetic activity (increased PEP) underlying this effect, which could be attributed to sleep deprivation.

It can be postulated that other exogenous factors (i.e. other than sleep deprivation) instigated the differences in cardiac measures between group 1 and group 2. For instance, the conditions from which subjects of group 1 and group 2 started in the constant routine experiment differed. Subjects of group 1 woke up in the sleep laboratory and were immediately exposed to the protocol. In contrast, subjects of group 2 were ambulatory (i.e. engaged in normal daily activities) during the time group 1 participated in the constant routine experiment. The discrepancy in activity could also have caused the observed group differences in the cardiac measures. IBI and RSA, however, showed no between-group differences during the period 2300–0500 hours, but patterns started to diverge during the morning hours (0800 hours and 1100 hours) when prior wakefulness was still equivalent. The between-group differences were most evident though during the diurnal hours when sleep deprivation was extended with 24 h in group 2 as measured up to group 1. Therefore, the effect of prior wakefulness could account for the sinusoidal × group interactions as have been found in these cardiac measures, possibly confounded by 'workday' recovery as well as non-defined psychological factors. Conversely, PEP only showed marked between-group differences during the period when prior wakefulness was equivalent. Thus, other exogenous factors (i.e. other than sleep deprivation) could be accountable for the sinusoidal × group interactions as have been found in PEP.

Based on the data obtained from group 1, it is nonetheless concluded that a circadian rhythmicity exists in base rate parasympathetic (vagal) modulation of heart rate that is most likely endogenous (i.e. regulated by the SCN). Based on the observed differences between groups, it is concluded that exogenous parasympathetic (vagal) modulation of cardiac activity also exists, probably caused by both prior wakefulness and other exogenous factors such as prior activation. With regard to sympathetic modulation, on the other hand, any endogenous

circadian influence seems to be absent or masked by the other exogenous factors such as prior activation.

These conclusions, however, should be interpreted with caution. In fact, the multilevel analyses showed the contribution of the circadian pacemaker in IBI and RSA, but not in PEP, to be statistically significant. Nonetheless, the low standardized β-values revealed small variance, which was likely caused by large between-subject variation. Future research should employ a larger study sample to be able to take the random factors of multilevel analysis into account. This way, more knowledge on the endogenous and exogenous factors underlying base rate cardiac activity and its mediation by the parasympathetic and sympathetic nervous system can be obtained.

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