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Circadian Variation in Cardiac Autonomic Activity: Reactivity Measurements to Different Types of Stressors

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ABSTRACT

The role of endogenous circadian rhythmicity in autonomic cardiac reactivity to different stressors was investigated. A constant routine protocol was used with repeated exposure to a dual task and a cold pressor test. The 29 subjects were randomly divided into two groups in order to manipulate prior wakefulness. Group 1 started at 09:00 h immediately after a monitored sleep period, whereas group 2 started 12 h later. Measures of interbeat intervals (IBI), respiratory sinus arrythmia (RSA, a measure of parasympathetic activity), pre-ejection period (PEP, a measure of sympathetic activity), as well as core body temperature (CBT) were recorded continuously. Multilevel regression analyses (across-subjects) revealed significant (mainly 24 h) sinusoidal circadian variation in the response to both stressors for IBI and RSA, but not for PEP. Individual 24 + 12 h cosine fits demonstrated a relatively large interindividual variation of the phases of the IBI and RSA rhythms, as compared to that of the CBT rhythm. Sinusoidal by

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group interactions were found for IBI and PEP, but not for RSA. These findings were interpreted as an indication for endogenous circadian and exogenous parasympathetic (vagal) modulation of cardiac reactivity, while sympathetic reactivity is relatively unaffected by the endogenous circadian drive and mainly influenced by exogenous factors.

Key Words: Stress reactivity; Parasympathetic activity; Sympathetic activity; Heart rate; Constant routine; Circadian rhythmicity.

INTRODUCTION

The aim of the current study was to investigate the influence of endogenous circadian rhythmicity (originating from the suprachiasmatic nucleus, SCN) in autonomic cardiac reactivity to different types of stressors. Although evidence of the role of the SCN in variation of base rate cardiac activity has been established in animal studies (Scheer et al., 2001; Warren et al., 1994) and strongly suggested in humans (Scheer et al., 1999), circadian reactivity studies are still scarce and inconclusive. Thus, more insight in circadian modulation of cardiac reactivity and the underlying autonomic processes is needed and of importance for understanding the 24 h patterns in cardiac events such as angina pectoris attacks (Nademanee et al., 1987) and transient myocardial ischemia (Rocco et al., 1987).

Nebel and colleagues (1996) conducted a study in which subjects were submitted to the same mental and physical stressors twice a day (i.e., at 07:30 h and 12:30 h) to assess time-of-day dependent variation in cardiovascular responses. They hypothesized that heart rate and blood pressure reactivity would be higher during the morning session. Their results, however, failed to show such differences. Nonetheless, other cardiac reactivity studies, performed by Adan and Sánchez-Turet (1996; 2001), have shown indications of a time dependency of heart rate reactivity to mental stressors. Adan and Sánchez-Turet have measured cognitive task performance of their subjects hourly between 08:00 and 21:00 h. Their data have demonstrated that cardiac reactivity only could be observed at certain times (i.e., at the start and the end of their recording period and around 15:00 h). However, in these studies endogenous circadian rhythmicity in cardiac reactivity has not been properly demonstrated.

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 (1978) and later modified by Czeisler and colleagues (1985). In this protocol, the evoked effects of changes in exogenous environmental (e.g., meal size) and behavioral (e.g., changes in posture) conditions are eliminated or evenly distributed across the 24 h period, thus enhancing the reliability of the assessment of the core circadian pacemaker characteristics and its direct contribution to rhythmicity in different variables (Duffy and Dijk, 2002; see also Kerkhof and van Dongen, 1996; Kerkhof et al., 1998; van Dongen et al., 2001; Varkevisser and Kerkhof, 2003). The current study made use of a constant routine protocol, with repeated exposure to stressors added in order to effectively study autonomic cardiac reactivity as a function of the circadian pacemaker.

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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) (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 (09:00 h) group and a late-start (21:00 h) group. In this way, the potential contributions of endogenous circadian rhythmicity and sleep deprivation to cardiac reactivity could be estimated.

Berntson and colleagues (1991; 1994) have introduced the concept of a two-dimensional (cardiac) autonomic reactivity plane. This plane visualizes task-dependent instances of co-activation or co-inhibition of the sympathetic and parasympathetic branches of the autonomic nervous system. Cardiac autonomic control cannot be characterized by a specific point on a continuum extending from sympathetic to parasympathetic dominance, since these axes may demonstrate independent reactivity to different tasks (Berntson et al., 1994; Cacioppo et al., 1993; 1994). An increase in heart rate (e.g., related to a brief laboratory stressor) may be associated with an increased sympathetic nervous system activation, a parasympathetic nervous system withdrawal, or both. To our knowledge, no studies have yet reported endogenous circadian rhythmicity in sympathetic and parasympathetic cardiac reactivity separately. The current study attempted to do just this.

Two types of stressors were used in this study that may differ in sympathetic and/or parasympathetic response: a physical challenge (cold pressor test) and a cognitive performance task (dual task). The cold pressor test (a brief immersion of one hand in ice water) is a well-tolerated brief aversive stressor that is known to increase the activity of the sympathetic nervous system by activation of both thermal and nociceptor afferents (Kelly and Cooper, 1998). The dual task is considered as a demanding task that is often used for the assessment of mental workload (e.g., Wetherell, 1990). This task is known to elicit both sympathetic activation and parasympathetic inhibition (Backs, 1998). Repeated application of these different stressors under constant routine conditions facilitates a comprehensive investigation into the circadian rhythmicity of endogenous cardiac reactivity.

MATERIALS AND METHODS

Subjects

The subjects, 33 Dutch students of the University of Leiden (15 M, 18 F, mean age 21.8 ± 4.1 SD years, BMI $= 21.7 \pm 2.0$ SD kg/m^2) were recruited through advertising and were paid for their contribution to the experiment. Ambulatory sleep/wake cycles were assessed two weeks prior to the experiment by means of a logbook. Mean bed-in and bed-out times were 00:39 h ($\pm$58 min SD) and 09:17 h ($\pm$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, BMI, 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 three 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 ($\geq$5 cigarettes), caffeine users ($\geq$350 mg/day), alcohol users ($\geq$5 standard drinks/week), and physical exercisers (>4 h a week) were excluded from the study.

Experimental Design

To assess the relative contribution of the endogenous circadian system to changes in cardiac reactivity, 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 lux, 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 ensure 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 09:00 until 12:00 h the next day. The second group contained 15 subjects who started 12 h later at 21:00 h until 00:00 h the next day.

Task Description

In the protocol of the current study, a task battery that consisted of four computerized cognitive performance tasks and a cold pressor test was presented every 3 h to monitor different types of performance as a function of time of day. The tasks were a mental arithmetic task, a working memory task (N-back), a signal detection task, and a dual task (unstable tracking and memory search). In addition to the cold pressor test results, only the results of the dual task will be presented here, as it was expected to have the strongest impact of all cognitive performance tasks. In dual task situations, it is the intention to force the subject to allocate resources between concurrent tasks in such a way that task demands exceed the available capacity of these recourses, which leads to performance decrement despite the effort to perform at a maximum level.

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1. Dual Task

The dual task was generated for computerized application with a code generating system (Experimental Run Time System, ERTS^{TM}) and was presented on a 15-inch, monochrome computer screen. The dual task combined an unstable tracking task with a memory search task. Each session started with 20 sec rehearsal, followed by the actual task. The tracking task was performed continuously because the subjects were required to constantly manipulate a joystick to nullify the position error between a target and a cursor. The memory task was applied with one level of memory load for probing short-term memory functions and consisted of four randomly selected digits (1–9) presented on the screen to be memorized before onset of the task. After the start of the task, the probes of the memory task were shown at the upper field of the screen within the focal field of the subject (1.2° above fixation). The subject had to indicate whether or not the presented digit belonged to the memory set by pressing one of the two specified keys: Yes for seen previously in the series of four digits, or No, not seen earlier.

Presentation time for the probes was 1500 ms or could be shortened by the subjects' response (self-paced). Immediately after each response (or 1500 ms), the probe digit on the screen was erased and a new probe was displayed after a fixed-response stimulus interval (RSI) of 400 ms. The time window in which responses were recorded was 1900 ms, i.e. during presentation time and RSI. The self-paced mode of memory search presentation allowed approximately an average of 150 probe stimuli to be presented during one task and determined the duration of approximately 3 min of the dual task. Both subtasks were shown continuous. Subjects used the dominant hand for tracking control, and the other hand for memory search responses.

2. Cold Pressor Test

The cold pressor test consisted of a brief immersion of one hand in ice water and may be considered as a brief physiological stressor. The cold pressor test used in this experiment consisted of a 3–5°C icebath, composed of 8 L of tap water and 4 L of crushed ice held in a plastic foam container. Before the test started, the ice was dissolved into water until the desired temperature was reached. The container was placed adjacent to the bed at the height of the subject's pelvis. The subject was then asked to submerge the non-dominant hand up to the wrist joint and to hold the fingers in a relaxed position. During the submersion, a small tube connected to an air pump was immersed into the water at the side of the container. This allowed the water to circulate gently to secure cooling of the hand. After exactly 60 sec the hand was removed from the bath and immediately wrapped and dried by the experimenter.

Procedure

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

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

After the screening and consent procedures, all subjects came in the laboratory facility the first evening at 22:00 h, where they were informed on four cognitive computer tasks and instructed to perform on three practice trials of each task. Subjects then slept in the sleep laboratory to get adapted to the experimental environment (Agnew et al., 1966; Mendels and Hawkins, 1967). 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 22:00 h for a sleep registration night. Polysomnographic, cardiac, and core body temperature measurements were collected during the sleep period. The habitual bedtime and waketime of each subject were taken into account within the range of 23:30–00:30 h (lights off) and 07:30–08:30 h (lights on). At 09:00 h, subjects of group 1 assumed a semi-recumbent position on a bed (a metal rack supported the mattress to an angle of 45 degrees as back support) to which they were confined for the next 27 h, with the exception of toilet requirements. The first two hours of the constant routine procedure were considered adaptation hours. The subjects remained connected to the cardiac and temperature devices at all times. After waking up between 07:30–08:30 h, 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 20:00 h and were again connected to the cardiac and the temperature devices. At 21:00 h, they also began their constant routine procedure.

The task battery and the cold pressor test were presented at 11:00 h (or at 23:00 h in group 2) and was repeated every 3 h throughout the experiment. Duration of each computer task was approximately 3–4 min, and 1 min for the cold pressor test. After each task, the subject was asked to pause, sit still, and refrain from any activity for a period of 5 min. The order of the four computer tasks was randomized within and between subjects. The duration of the entire task battery was approximately 45 min.

Every hour, subjects were allowed to choose one 100–120 kcal snack from a list of 20, accompanied by 150 mL of water or sugar and caffeine-free soda. The consumption of the hourly snacks occurred on a ''must-finish'' basis. 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 (dZ) 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, Willemsen, Klaver and van Doornen (de Geus et al., 1995). Cross-instrumental comparison of the VU-AMS with a standard laboratory measurement setup showed excellent between-subjects and within-subjects 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 IBI: 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 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 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

1. Data Reduction

Base rate data collected just prior to the onset of a task battery (5 min), the data collected during the occurrence of the dual task (3 min) and the cold pressor test (1 min), and the data collected during the 5 min rest period immediately after the stressors were taken from the continuous cardiac dataset for reactivity analysis (see below).

The heart period (IBI) data of each participant were analyzed in segments representing 32 sec. An artifact pre-processing was performed on these segments by detecting outlier IBI values with two methods: (a) 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 sec) were subjected to a discrete Fourier transformation. Heart period power values were computed for the high-frequency (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

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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 base rate, stressor, and rest data period to obtain reliable time series estimates of cardiac reactivity. Note that the duration of the task battery resulted in a time difference between the base rate periods and the measurements during the dual task and the cold pressor test. Therefore, for each particular task, pre-task base rate values and post-task resting values were averaged and subsequently subtracted from the task values to obtain the cardiac reactivity scores, denoted as ΔIBI, ΔRSA, and ΔPEP.

CBT was analyzed on a 6 min basis. Outliers (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 data 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 base rate cardiac data, was used for further analysis (see below).

2. Multi-level (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 and 24 h sinusoidal fluctuations across subjects a regression analysis was employed on the cardiac reactivity data and base rate 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 differentiating between two types of variance: between-individual variance and within-individual variance, taking into account the dependencies that are present in the data and adjusting the standard errors of the estimated regression coefficients accordingly (Hox, 2002; Rasbash et al., 2000). 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 $\sin(a+b) = \sin(a)\cos(b) + \cos(a)\sin(b)$ to $f(t) = X1 \sin(2\pi t/24) + X2 \cos(2\pi t/24) + X3 \sin(2\pi t/12) + X4 \cos(2\pi t/12) + c$; 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: $\sin(2\pi t/24)$, $\cos(2\pi t/24)$, $\sin(2\pi t/12)$ en $\cos(2\pi t/12)$. Thus, the multilevel regression formula, with the two-level subjects and time number, is:

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

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Four beta values (fixed factors) were obtained that represent the (across-subjects) 12 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, and four additional terms (b_6–b_9) were modeled representing the Group by 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).

3. Individual Fit Procedure

Circadian rhythmicity in each individual physiological data profile was modeled (to explore individual variation) by fitting the superposition of a combined 24 h fundamental and a 12 h harmonic sinusoidal curve with custom-made software, using the formula:

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

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 ($c \approx 0$). The order of the computer tasks was randomized between subjects as well as within subjects. Since duration of the computer task section of the performance battery was 40 min, the mean time of dual task occurrence was set to 20 min past onset of the battery. The cold pressor test, on the other hand, was presented consistently at the end of the task battery. Therefore, the time of cold pressor test occurrence was fixed to 45 min past onset of the task battery. From the individual fits, range ($= t_{max} - t_{min}$), maximum value ($= t_{max}$), and mean value ($= t_{mean}$, since sampling was regular) were calculated. For the base rate CBT fits, the minimum value ($= t_{min}$) was calculated. Since two frequencies were combined, the phase estimation and range were determined by using the absolute minimum and maximum of the interpolated curve. For the individual temperature profiles, the determination of fits was done over the initial sample frequency for a period of 24 h (= 240 samples).

RESULTS

Mean (across subject) Core Body Temperature

The mean core body temperature values for the two groups have been plotted in Fig. 1. For both groups a distinct and significant 24 h sinusoidal pattern is shown (see Table 1), with maximal values around 20:00 h and minimal values around 05:00 h. No Group effects or Sinusoidal × Group interactions were found. 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). However, the expected 12 h main Sinusoidal effect was not obtained (Table 1).

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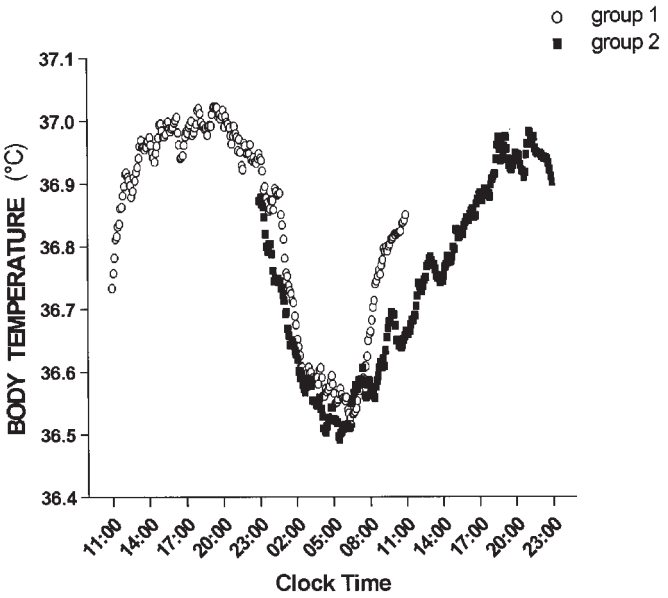

Figure 1. 24 h profile of core body temperature for group 1 and group 2.

Table 1. Standardized β-values of the multilevel regression analysis. 24 and 12 h sinusoidal components were modeled with ΔIBI, ΔRSA, ΔPEP, and core body temperature measurements. Subsequently, group effects and the interaction of the factor group with sinusoidal effects are stated.

	Dual task			Cold pressor test			
	ΔIBI	ΔRSA	ΔPEP	ΔIBI	ΔRSA	ΔPEP	CBT
Sinusoidal effects							
cosinus 24 h	.17*	.14	−.04	.06	.01	.08	.04
sinus 24 h	−.16*	−.15*	−.01	−.14*	−.16*	−.04	.45***
cosinus 12 h	.03	.06	−.11	.09	<.01	<.01	.08
sinus 12 h	−.09	.05	.04	−.11	−.17*	−.03	−.02
Group effects	<.01	−.11	<.01	−.10	.02	−.02	−.13
Sinusoidal × Group effects							
cosinus 24 h	−.11	−.02	.04	.13	.11	.02	.07
sinus 24 h	.18*	.07	.26**	.13*	−.03	.19*	.05
cosinus 12 h	−.02	−.08	.13	<.01	.05	−.03	−.03
sinus 12 h	.21*	.08	.01	.09	.12	<.01	−.05

Asteriscs correspond to the level of significance: $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$.

Mean (across-subjects) Cardiac Reactivity

1. IBI

The mean ΔIBI values for the dual task and the cold pressor test across the subjects of the two groups are shown in Figs. 2a and 3a, respectively. For both tests, the subjects of group 1 reached a minimum reactivity score in response to the 20:00 h session, and a maximum following the 05:00 h session. For group 2, however, the time courses of the scores for the two tests differed substantially. Whereas the first halves of their 24 h patterns show a similar increase in reactivity, corresponding with that of group 1, the later halves follow opposite courses: an increase in reactivity to the dual task vs. a decrease in reactivity to the cold pressor test. For both tests significant Sinusoidal effects (24 h sinus and 24 h cosinus, $p < 0.05$) were obtained, significant Sinusoidal × Group effects significance of some results, the standardized β-values for ΔIBI remained relatively small (range: −0.16 to 0.21). Also, it should be noted that the mean ΔIBI score for the cold pressor test fluctuates around −100 ms, in contrast to that for the dual task (−10 ms).

2. RSA

The mean ΔRSA values for the dual task and the cold pressor test across the subjects of the two groups are shown in Figs. 2b and 3b, respectively. The subjects of group 1 reached a minimum reactivity score around 20:00 h and a maximum around 05:00 h for both tests. For group 2, however, the time courses of the ΔRSA scores for the two tests differed substantially. Whereas the first halves of their 24 h patterns show a similar increase in reactivity, corresponding with that of group 1, the later halves follow opposite courses: a modest increase in reactivity to the dual task vs. a decrease in reactivity to the cold pressor test. For both tests significant Sinusoidal effects were obtained (dual task: 24 h sinus, $p < 0.05$, and the cold pressor test: 24 h sinus and 12 h sinus, $p < 0.05$, respectively). No Group effects or Sinusoidal × Group effects were found (see Table 1). Notify that despite the significance of some results, the standardized β-values for ΔRSA also remained relatively small (range: −0.15 to −0.17). Also, note that the level difference between the two tests observed in ΔIBI is not seen in ΔRSA.

3. PEP

The mean ΔPEP values for the dual task and the cold pressor test across the subjects of the two groups are shown in Figs. 2c and 3c, respectively. For both tests, the subjects of group 1 show no characteristic pattern, fluctuating around −2 ms. For group 2, the latter halves of their 24 h pattern follow opposite courses: an increase in reactivity to the dual task vs. a decrease in reactivity to the cold pressor test. No significant Sinusoidal effects or Group effects were obtained. However, Sinusoidal × Group effects (dual task: 24 h sinus, $p < 0.01$, and the cold pressor

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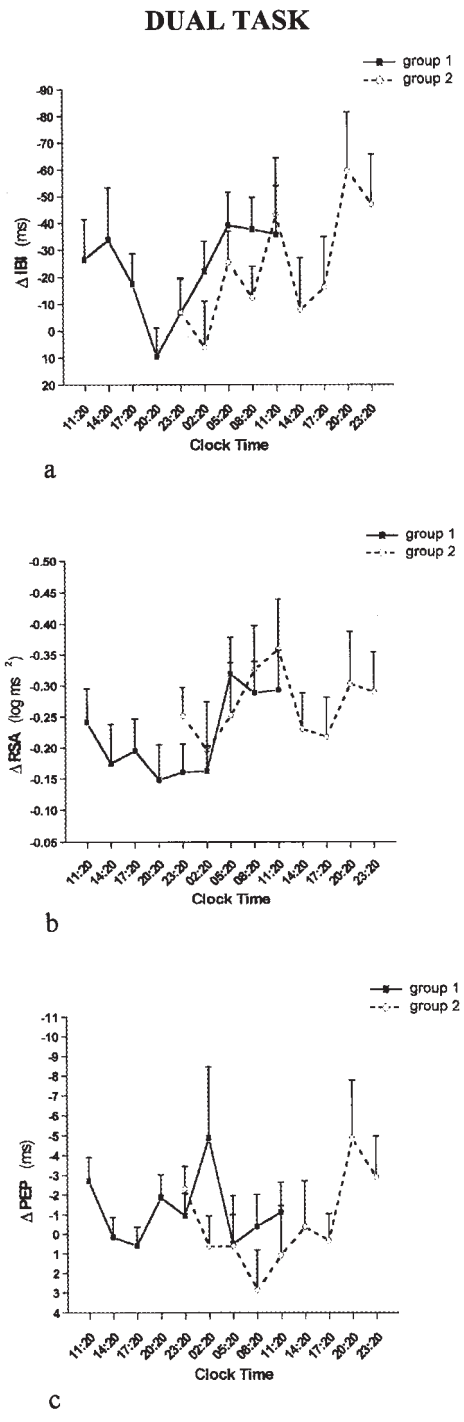

Figure 2. IBI, RSA, and PEP reactivity measures as a result of dual task exposure for group 1 and group 2. Bars represent standard error of mean.

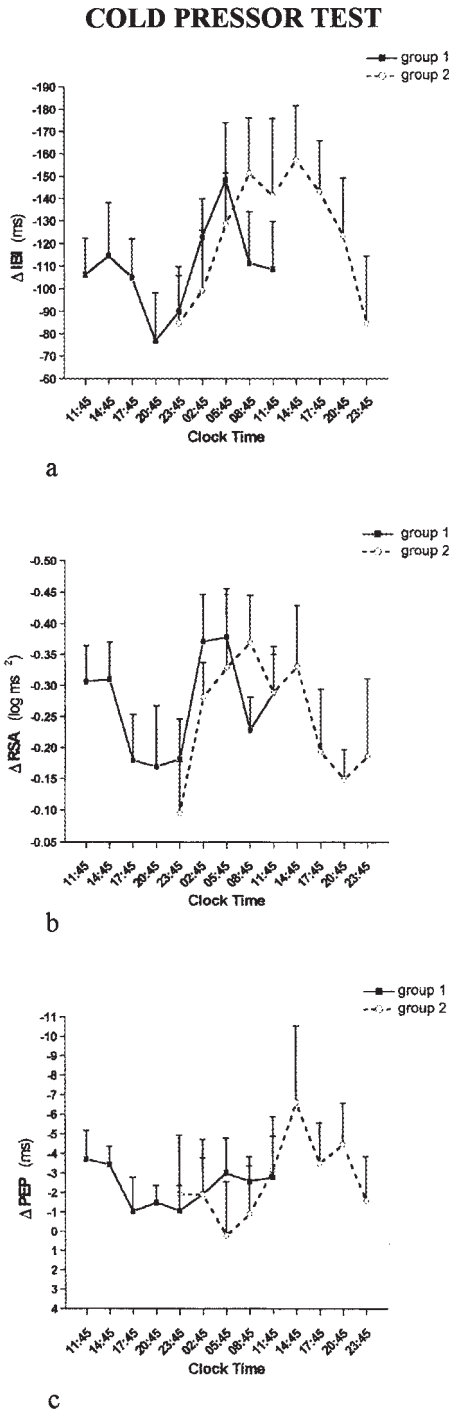

Figure 3. IBI, RSA, and PEP reactivity measures as a result of cold pressor test exposure for group 1 and group 2. Bars represent standard error of mean.

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test: 24 h sinus, $p < 0.05$, respectively) were found. Note that despite the significance of some results, the standardized β-values for ΔPEP also remained relatively small (range: .19 to .26). Also, note that the level difference between the two tests observed in ΔIBI is not seen in ΔPEP.

Individual Fits

The parameters obtained from the individual profile fits, modeled by means of a 24 + 12 h cosine fit are shown in Tables 2 and 3. Phase, mean value, and range are listed for all subjects of both groups, accompanied by quality conditions of the fit (i.e., correlation and level of significance).

Core Body Temperature

The core body temperature showed a relatively large across-subject similarity, regardless of group. As seen in Tables 2 and 3, the mean fluctuated around 36.8°C with a range of about 0.9°C in group 1 and around 36.7°C with a range of about 0.8°C in group 2. Between-group comparisons revealed no significant differences in all fit parameters ($p > 0.05$).

Cardiac Reactivity

A majority of the subjects showed significant fits for the cardiac reactivity measures, regardless of group and type of stressor. However, the phase estimations showed large standard deviations (across subjects) as compared to the standard deviations of the CBT fits, excepting the phase estimation of ΔPEP in group 2 for the cold pressor test.

Group differences in fit parameters were tested nonparametric using the Mann-whitney test. No significant group differences were found between the mean values and the ranges for ΔIBI and ΔRSA, except for ΔPEP range ($Z = -2.043$, $p = 0.041$) related to the cold pressor test exposure. However, significant group differences in phase estimations were found for ΔIBI ($Z = -2.770$, $p = 0.006$), ΔRSA ($Z = -1.958$, $p = 0.050$), and ΔPEP ($Z = -4.320$, $p < 0.001$) related to the dual task, and for ΔIBI ($Z = -3.536$, $p < 0.001$), ΔRSA ($Z = -3.154$, $p = 0.002$), and ΔPEP ($Z = -4.319$, $p < 0.001$) related to the cold pressor test.

DISCUSSION

The current study, performed to demonstrate endogenous circadian rhythmicity in autonomic cardiac reactivity to different stressors, obtained three primary results. First, based on the chronological plotted cardiac reactivity data (i.e., Figs. 2a–3c) and the results from the multilevel analyses, heart rate, and cardiac vagal reactivity showed a 24 h sinusoidal rhythmicity (similar to base rate CBT) when monitored

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during the initial 24 h of the constant routine (group 1). This rhythmicity was very similar for the dual task and the cold pressor test. Sympathetic activity, however, failed to mirror these results. Second, an extension of prior wakefulness with 12 h (group 2) resulted in a 24 h pattern for heart rate and sympathetic reactivity, which differed between the two tests. Third, large interindividual variations were found in the observed cardiac reactivity measures, resulting in large across-subject phase convergences and small (fixed-factor) standardized β-values (as compared to base rate CBT).

The use of different types of stressors in the current study revealed some additional notable outcomes. First, the relatively large reduction in mean ΔIBI for the cold pressor test (-100 ms) as compared to the dual task (-10 ms) was not reflected in ΔRSA or ΔPEP. This result is in conflict with the two-dimensional (cardiac) autonomic reactivity plane (Berntson et al., 1991; 1994), under the assumption that parasympathetic and sympathetic modulatory effects on heart rate are reflected in ΔRSA and ΔPEP. Thus, other cardiac modulatory effects must have been responsible for this effect. For instance, Porges (1995) found that changes in heart rate as a response to stress and exercise may be caused by cardiac vagal pathways that are not reflected in RSA.

Second, the dual task reactivity measures in group 2 showed an *increase* during the afternoon and evening while, in contrast, cold pressor test reactivity in group 2 showed a *decrease*. These opposite trends occurred only after >24 h of sleep deprivation. An important difference between these stressors is the demand of mental resources. It is known that (dual) task performance, which requires mental capacity, is sensitive to sleep loss (Dinges, 1992). Thus, the increase in cardiac reactivity to the dual task may be a result of mounted effort needed to maintain the execution of the task despite of sleepiness and fatigue. In contrast, cardiac reactivity to the cold pressor test may be insensitive to the accumulation of sleep loss, because this test can be referred to as a physical challenge with only a passive coping component.

The primary results of the current reactivity study show remarkable similarities with recent work that examined potential circadian influences on base rate cardiac autonomic activity via use of the constant routine (Burgess et al. 1997; Kräuchi et al., 2000). In the latter study, heart rate and vagal measures demonstrated an (endogenous) circadian rhythmicity in base rate activity, while sympathetic measures did not. Also, Kräuchi et al. (2000) concluded that heart rate is mainly under the control of vagal activity. Additionally, the exogenous effect caused by our extension of prior wakefulness with 12 h (group 2) resulted in a different circadian pattern for heart rate and sympathetic reactivity measures. Thus, endogenous circadian and exogenous factors underlie parasympathetic (vagal) modulation of base rate cardiac activity and cardiac reactivity to stress, while sympathetic modulation of base rate cardiac activity and cardiac reactivity is mainly influenced by exogenous factors. There are two possible approaches for interpretation of these results: (I) Independent endogenous circadian modulation exists on both cardiac base rate activity and cardiac reactivity and (II) Endogenous circadian modulation of cardiac base rate activity levels enhance or inhibit the mechanism's sensitivity to stress. Thus, cardiac reactivity as a function of time may be secondary to base rate cardiac activity, i.e., accounted for by the Law of Initial Values (LIV). The LIV asserts that the

Table 2. Individual profiles of 24 + 12h cosine fit parameters for base rate core body temperature and cardiac reactivity to the dual task. In case of the cardiac reactivity measures, the level of significance was set to a probability of < .1, due to the limited number of data points. Missing temperature data due to equipment failure is marked by xxx.

	Δ Interbeat interval				Δ Respiratory sinus arrythmia				Δ Pre-ejection period				Core body temperature			
Subjects	Phase min (h)	Mean (ms)	Range (ms)	Correlation	Phase min (h)	Mean (ms)	Range (log ms^2)	Correlation	Phase min (h)	Mean (ms)	Range (ms)	Correlation	Phase min (h)	Mean (°C)	Range (°C)	Correlation
Group 1																
1	10:13	13	35	.615*	8:51	−.14	.26	.392 n.s.	13:24	2	9	.675**	xxx	xxx	xxx	xxx
2	7:16	−12	82	.741**	5:14	−.14	.25	.613*	18:12	−1	1	.306 n.s.	5:05	36.77	.64	.971***
3	22:57	−37	88	.897***	21:43	−.25	.35	.769**	15:19	1	2	.605*	1:22	36.93	.64	.887***
4	7:25	−29	72	.851***	7:24	−.04	.31	.725**	12:56	0	4	.712**	2:06	36.87	1.50	.938***
5	11:58	−12	32	.421 n.s.	5:27	−.43	.43	.904***	14:07	0	7	.726**	6:47	37.21	1.00	.866***
6	10:46	−42	140	.763**	10:09	−.22	.56	.854***	0:17	−4	31	.877***	4:18	37.14	.88	.726***
7	13:07	−51	80	.547 n.s.	3:50	−.32	.32	.444 n.s.	22:55	−2	9	.781**	2:23	36.58	.73	.813***
8	2:54	−29	178	.725**	5:57	−.16	.38	.672**	4:55	−3	6	.937***	6:31	36.76	1.27	.825***
9	6:42	9	82	.719**	6:50	−.13	.37	.666**	8:10	−4	12	.691**	5:10	36.68	.81	.946***
10	4:35	−45	89	.625*	10:29	−.11	.53	.803***	1:58	−2	2	.658*	5:43	36.71	.70	.923***
11	8:21	−31	65	.677**	7:34	−.34	.34	.692**	7:37	0	12	.852***	7:47	37.28	.63	.948***
12	5:22	−65	263	.801***	7:13	−.49	.69	.875***	20:22	2	19	.837***	6:16	36.63	1.21	.975***
13	15:40	−35	53	.540 n.s.	10:24	−.23	.20	.347 n.s.	21:16	0	13	.904***	xxx	xxx	xxx	xxx
14	7:32	−6	124	.770**	7:12	−.11	.27	.645*	6:56	−5	10	.856***	6:11	36.52	.63	.903***
15	4:43	11	131	.748**	9:20	−.11	.28	.687**	17:03	4	6	.628*	5:48	36.57	.70	.889***
mean (sd)	6:14	−22	112		6:43	−.21	.40		22:15	−1	10		5:02	36.82	.87	
of sig. fits	(3:14)	(25)	(62)		(3:18)	(.14)	(.13)		(6:58)	(3)	(8)		(1:57)	(.25)	(.29)	

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Group 2																
1	0:45	−61	85	.369 n.s.	4:14	−.46	.90	.795**	15:26	2	17	.849***	xxx	xxx	xxx	xxx
2	19:41	−13	143	.882***	7:57	−.42	.50	.774**	20:09	1	9	.724**	4:48	36.63	.70	.891***
3	0:07	−40	73	.743**	10:36	−.08	.20	.838***	19:13	−5	13	.719**	4:52	36.48	1.03	.951***
4	12:39	−58	98	.632*	10:21	−.22	.59	.934***	19:51	−7	20	.634*	9:03	36.82	.80	.938***
5	7:36	22	89	.485 n.s.	1:19	−.08	.16	.338 n.s.	20:32	0	23	.826***	3:20	37.30	.55	.797***
6	11:22	−27	127	.781**	9:12	−.53	.43	.674**	16:40	12	32	.779**	3:22	36.68	.68	.904***
7	19:07	−16	160	.842***	9:58	−.20	.47	.924***	12:47	0	3	.434 n.s.	4:45	36.33	.94	.961***
8	20:03	27	114	.858***	6:47	−.15	.27	.874***	14:45	−3	4	.683**	2:46	36.79	.89	.928***
9	17:37	−94	69	.634*	5:08	−.69	.65	.931***	14:44	0	3	.857***	3:38	36.83	.81	.895***
10	17:29	−62	186	.900***	19:20	−.35	.99	.945***	17:43	−3	9	.808***	7:51	36.73	.64	.856***
11	4:04	−10	54	.738**	21:54	.05	.19	.920***	10:57	−4	5	.426 n.s.	4:11	36.74	.81	.920***
12	11:31	−13	87	.878***	11:48	−.21	.48	.831***	16:33	2	33	.961***	8:13	36.89	.90	.926***
13	21:37	−41	279	.733**	12:27	−.27	.35	.693**	13:50	−6	15	.807***	8:48	36.80	.63	.923***
14	2:03	−15	60	.482 n.s.	8:50	−.15	.23	.779**	20:27	1	21	.911***	3:35	36.53	1.00	.940***
mean (sd)	14:07	−32	126		10:39	−.28	.48		17:31	−1	15		5:19	36.73	.80	
of sig. fits	(6:57)	(32)	(65)		(5:03)	(.20)	(.25)		(2:27)	(5)	(10)		(2:17)	(.23)	(.15)	

Asteriscs correspond to the level of the significance of the fit: $^{*}p < .1$, $^{**}p < .05$, $^{***}p < .01$.

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Table 3. Individual profiles of 24 + 12h cosine fit parameters for base rate core body temperature and cardiac reactivity to the cold pressor test. In case of the cardiac reactivity measures, the level of significance was set to a probability of < .1, due to the limited number of data points. Missing temperature data due to equipment failure is marked by xxx.

	Δ Interbeat interval				Δ Respiratory sinus arrythmia				Δ Pre-ejection period				Core body temperature			
Subjects	Phase min (h)	Mean (ms)	Range (ms)	Correlation	Phase min (h)	Mean ($\log ms^2$)	Range ($\log ms^2$)	Correlation	Phase min (h)	Mean (ms)	Range (ms)	Correlation	Phase min (h)	Mean (°C)	Range (°C)	Correlation
Group 1																
1	7:44	−58	52	.759**	13:46	−.05	.67	.906***	13:19	1	9	.866***	xxx	xxx	xxx	xxx
2	15:31	−158	111	.651*	11:17	−.11	.29	.601*	4:27	−3	3	.763**	5:05	36.77	.64	.971***
3	1:18	−49	136	.844**	22:23	−.20	.37	.599*	15:19	0	5	.879***	1:22	36.93	.64	.887***
4	7:08	−58	216	.828*	7:44	−.09	.30	.813***	18:06	−5	8	.623*	2:06	36.87	1.50	.938***
5	16:08	−142	173	.787**	1:55	−.37	.77	.974***	23:24	−3	13	.784**	6:47	37.21	1.00	.866***
6	8:03	−51	281	.909***	3:02	−.07	1.04	.959***	9:07	−2	17	.542 n.s.	4:18	37.14	.88	.726***
7	5:01	−151	207	.909***	5:00	−.36	.79	.977***	13:15	−6	15	.663*	2:23	36.58	.73	.813***
8	2:57	−90	150	.818***	3:06	−.14	.43	.717**	3:24	−4	5	.880***	6:31	36.76	1.27	.825***
9	4:01	−109	124	.892***	2:44	−.21	.13	.323 n.s.	4:27	−7	20	.863***	5:10	36.68	.81	.946***
10	14:42	−99	164	.922***	14:29	−.26	.98	.820***	2:32	−5	24	.687**	5:43	36.71	.70	.923***
11	11:11	−56	71	.359 n.s.	3:04	−.23	.40	.637*	19:28	−1	9	.865***	7:47	37.28	.63	.948***
12	6:00	−176	423	.922***	5:38	−.51	.93	.870***	20:05	3	12	.439 n.s.	6:16	36.63	1.21	.975***
13	6:09	−112	65	.466 n.s.	13:02	−.40	.16	.318 n.s.	14:14	−1	7	.653*	xxx	xxx	xxx	xxx
14	6:54	−142	129	.632*	6:47	−.50	.79	.831***	8:52	−1	21	.819***	6:11	36.52	.63	.903***
15	3:27	−191	83	.782**	11:24	−.51	.24	.658*	15:49	0	11	.707**	5:48	36.57	.70	.889***
mean (sd)	2:04	−113	173		2:53	−.26	.62		21:17	−3	12		5:02	36.82	.87	
of sig. fits	(6:22)	(50)	(96)		(6:43)	(.17)	(.29)		(6:51)	(3)	(7)		(1:57)	(.25)	(.29)	

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Group 2																
1	10:51	−192	124	.306 n.s.	6:59	−.53	1.16	.692**	21:19	−2	12	.659*	xxx	xxx	xxx	xxx
2	20:16	−30	157	.939***	13:39	−.19	.51	.902***	8:35	0	24	.952***	4:48	36.63	.70	.891***
3	14:25	−180	126	.625*	8:58	−.20	.69	.757**	20:21	−10	9	.649*	4:52	36.48	1.03	.951***
4	14:09	−85	212	.866***	6:44	−.22	.60	.659*	21:36	−4	11	.702**	9:03	36.82	.80	.938***
5	6:28	−126	94	.827***	3:57	−.16	.18	.322 n.s.	16:33	6	30	.854***	3:20	37.30	.55	.797***
6	17:41	−116	71	.594*	8:52	−.32	.52	.683**	15:21	6	26	.705**	3:22	36.68	.68	.904***
7	6:40	−225	203	.704**	15:42	−.34	.40	.643*	12:26	−11	19	.881***	4:45	36.33	.94	.961***
8	20:48	−102	200	.803***	9:17	−.18	.99	.979***	16:24	0	16	.686**	2:46	36.79	.89	.928***
9	14:50	−152	29	.264 n.s.	13:48	−.25	.56	.846***	8:49	−1	9	.969***	3:38	36.83	.81	.895***
10	15:14	−146	183	.946***	6:14	−.23	.72	.755**	12:55	−1	6	.771**	7:51	36.73	.64	.856***
11	5:48	−79	138	.782**	8:14	−.14	.67	.823***	9:01	−5	11	.733**	4:11	36.74	.81	.920***
12	13:48	−35	171	.749**	14:42	−.50	.70	.796**	13:46	−8	43	.715**	8:13	36.89	.90	.926***
13	11:36	−264	234	.783**	5:43	−.47	.50	.803***	20:01	−6	23	.716**	8:48	36.80	.63	.923***
14	18:53	−40	172	.783**	16:21	−.01	.54	.913***	10:38	2	21	.744**	3:35	36.53	1.00	.940***
mean (sd)	13:48	−119	163		10:24	−.28	.66		14:50	−2	19		5:19	36.73	.80	
of sig. fits	(5:17)	(74)	(49)		(3:51)	(.15)	(.21)		(4:42)	(5)	(10)		(2:17)	(.23)	(.15)	

Asteriscs correspond to the level of the significance of the fit: $^{*}p < .1$, $^{**}p < .05$, $^{***}p < .01$.

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magnitude in which a subject responds to a stressor, such as with an increase in heart rate, is dependent on the momentary value of cardiac activity (cf. Berntson et al., 1994). Because of the exploratory nature of the current study's design and the relative limited number of subjects, no further analyses and firm conclusions regarding this interpretation can be made.

Although our results are not completely in harmony with the two-dimensional (cardiac) autonomic reactivity plane (see introduction), they do indicate that task-related reactivity of heart rate is related to the sympathetic and parasympathetic branches of the autonomic nervous system (assessed here as ΔPEP and ΔRSA), and that these branches do not function as mere antagonists. Berntson (1994) stated that psychophysiological relationships involve two major classes of transformations: (a) from psychophysiological antecedents to autonomic outflows and (b) from autonomic outflows to functional effects on target organs. By quantifying autonomic space and its manifestations at target organs, variance in psychophysiological data associated with the second of these transformations can be specified. The results of the current study demonstrate that the activity of the SCN intervenes with the second transformation and could therefore be added as a third axis in the autonomic space model, representing the circadian influence on sympathetic and parasympathetic task-dependent modulation of heart activity. Comprehension on this source of variance may clarify or reveal psychophysiological relationships that would otherwise remain ambiguous.

Future research could take the current results into consideration for investigating the possible connection between time-dependent reactivity to stress and the time-matching occurrence of stress-related diseases (cf. Marler et al., 1989; Muller et al., 1985). Moreover, succeeding studies could make an attempt to separate endogenous and exogenous factors that mediate the variation of cardiac stress responses as a function of time, for instance by increasing statistical power to take random factors of the multilevel regression analysis into account.

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