Autonomic activity during human sleep as a function of time and sleep stage

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SUMMARY
While there is a developing understanding of the influence of sleep on cardiovascular autonomic activity in humans, there remain unresolved issues. In particular, the effect of time within the sleep period, independent of sleep stage, has not been investigated. Further, the influence of sleep on central sympathetic nervous system (SNS) activity is uncertain because results using the major method applicable to humans, the low frequency (LF) component of heart rate variability (HRV), have been contradictory, and because the method itself is open to criticism. Sleep and cardiac activity were measured in 14 young healthy subjects on three nights. Data was analysed in 2-min epochs. All epochs meeting specified criteria were identified, beginning 2 h before, until 7 h after, sleep onset. Epoch values were allocated to 30-min bins and during sleep were also classified into stage 2, slow wave sleep (SWS) and rapid eye movement (REM) sleep. The measures of cardiac activity were heart rate (HR), blood pressure (BP), high frequency (HF) and LF components of HRV and pre-ejection period (PEP). During non-rapid eye movement (NREM) sleep autonomic balance shifted from sympathetic to parasympathetic dominance, although this appeared to be more because of a shift in parasympathetic nervous system (PNS) activity. Autonomic balance during REM was in general similar to wakefulness. For BP and the HF and LF components the change occurred abruptly at sleep onset and was then constant over time within each stage of sleep, indicating that any change in autonomic balance over the sleep period is a consequence of the changing distribution of sleep stages. Two variables, HR and PEP, did show time effects reflecting a circadian influence over HR and perhaps time asleep affecting PEP. While both the LF component and PEP showed changes consistent with reduced sympathetic tone during sleep, their pattern of change over time differed.

KEYWORDS blood pressure, heart rate, sympathetic nervous system, parasympathetic nervous system, sleep onset, vagal tone

INTRODUCTION
Numerous studies have recorded human blood pressure (BP) and heart rate (HR) over 24 h, showing reductions during the nyctohemeral phase (e.g. Bevan et al. 1969; Mancia et al. 1983; Furlan et al. 1990; Parati et al. 1990). The periods of reduced cardiac activity have been shown to coincide with the occurrence of sleep (Bristow et al. 1969; Smyth et al. 1969).

Further, baroreflex sensitivity increases during sleep (Smyth et al. 1969; see also Lombardi and Parati, 2000 for a review of this literature). More recently, constant routine studies have indicated that the fall in HR during sleep is partly attributable to the circadian system (Burgess et al. 1997; Kerkhof et al. 1998). In contrast, the fall in BP is entirely because of the influence of sleep (Kerkhof et al. 1998).

Studies that have specifically investigated sleep in humans have confirmed that there is a fall in BP and HR during non-rapid eye movement (NREM) sleep as compared with relaxed wakefulness. Blood pressure may also be lower in slow wave sleep (SWS) than stage 2. During rapid eye movement (REM)
Sleep BP, and in some studies HR, return to waking levels. In
other studies HR remains below waking, but above NREM
sleep levels. During phasic REM sleep, BP and HR both show
pulsatile increases in activity (Zemaityte et al. 1984; van de
Borne et al. 1994; Morgan et al. 1996).

Sleep related changes in HR and BP appear to be mediated
primarily by changes in autonomic circulatory control
(Narkiewicz et al. 2000). Autonomic control of cardiac
activity in humans has been investigated using three tech-
niques: spectral analysis of BP and interbeat interval (IBI);
microneurographic studies of muscle sympathetic nerve
activity (MSNA) and pharmacological blockade. Several
effects have been consistently reported. The first is an
increase in vagal activity during NREM sleep (e.g. Furlan
et al. 1990; Berlad et al. 1993; Orr et al. 1993; Vanoli et al.
1995; Bonnet and Arand 1997; Burgess and Trinder 1996), an
effect that may (Negoeescu and Csiki 1989; van de Borne et al.
1994), or may not (Zemaityte et al. 1984; Vaughn et al.
1995), increase with the development of SWS. The second is a
reduction in total sympathetic vascular tone, as indicated by
a fall in muscle sympathetic nerve activity (Hornyk et al.
1991; Okada et al. 1991; Somers et al. 1993; Takeuchi et al.
1994) and a fall in the power of low frequency BP variability
(Furlan et al. 1990; van de Borne et al. 1994). Finally, during
REM sleep there is a reversal of these two changes (Hornyk
et al. 1991; Berlad et al. 1993; Somers et al. 1993; van de
Borne et al. 1994).

In contrast, measures of central sympathetic activity have
produced more variable results. Thus, the low frequency (LF)
component of heart rate variability (HRV) has been shown to
decrease (Berlad et al. 1993; van de Borne et al. 1994; Vanoli
et al. 1995; Vaughn et al. 1995; Bonnet and Arand 1997),
remain unchanged (Orr et al. 1993; Burgess and Trinder 1996),
or increase (Parati et al. 1990) during sleep. Further, an
alternative, non-invasive measure of central cardiac sympa-
thetic activity, pre-ejection period (PEP), has recently been
shown to increase (decreased sympathetic activity) during
sleep, but to have a different time course to the LF component
over the sleep period (Burgess et al. 1997, 1999). In addition to
variability in the data, there is dispute as to whether the LF
component does (Pagani et al. 1986; Malliani et al. 1994), or
does not (Berntson et al. 1997) reflect sympathetic nervous
system (SNS) activity.

Studies that have investigated the effect of sleep and sleep
stages on autonomic control have made the assumption that
stage effects are independent of time during the sleep period.
This is implicit in the typical methodology in which stages are
sampled by relatively short epochs selected independent of
time, restricted to a particular time such as the first sleep cycle,
or averaged over the whole night. However, the assumption
that the effect of sleep stage is independent of time may not be
valid, as it is already known that HR (Degaute et al. 1991;
Burgess et al. 1997; Kerkhof et al. 1998), and possibly vagal
activity (Burgess et al. 1997), are influenced by the circadian
system. Further, the data of a number of studies that have
conducted 24 h assessment of cardiac activity suggest varia-
tions in activity within the sleep period (Furlan et al. 1990;
Parati et al. 1990; Degaute et al. 1991; van de Borne et al.
1994).

As a consequence of these considerations the current study
was designed to investigate two aspects of autonomic circu-
latory control. The first was the effect of sleep and sleep stages
on cardiac autonomic activity, and in particular on two
measures of central cardiac sympathetic activity, the LF
component of HRV and PEP. The question asked with respect
to the two measures of SNS activity, was whether PEP would
provide convergent validation of the LF component. The
second issue to be investigated was the effect of time during
sleep, within stage of sleep. In addition to measures of
autonomic control, two measures of overall cardiac activity,
HR and BP were assessed.

METHODS

Subjects

Fourteen subjects (six female and eight male) with a mean age
of 21.4 years (range 18–25) and a mean body mass index
(BMI) of 22.7 (range 19–28) participated in the study. All
subjects were healthy and free of physical illness and were not
taking medication at the time of the study. Other exclusion
criteria included a personal or family history of a sleep
disorder, cardiovascular or respiratory problems, shiftwork or
trans-meridian travel in the past 3 months, heavy caffeine
(> 350 mg day−1), alcohol (>5 standard drinks per week) or
other recreational drug consumption and intense, regular
physical exercise. Female subjects were not selected if they
were taking an oral contraceptive, although menstrual phase
was not controlled. Finally, subjects were not run in the study
at times of major life stress, such as during examination
periods.

Design

Subjects were run in the laboratory on an adaptation night
and either two (four subjects) or three (10 subjects) experi-
mental nights. On each experimental night subjects were put
to bed 2 h before their normal sleep onset time. During this
period they were required to remain in a supine position with
their head slightly elevated and to remain awake. They were
permitted to read, listen to music, or watch TV. Both the
subjects and their ongoing sleep recordings were scrutinised
during the presleep period to ensure they did not go to sleep.
Lights were turned out and subjects requested to go to sleep
at their normal sleep onset time. They were then left
undisturbed until their normal waking time in the morning.
Cardiovascular activity was subsequently analysed during
both the 2 h of presleep wakefulness and the first 7 h of sleep.
During both periods the data was analysed as a function of
time in 30-min bins. Finally, during the sleep period the data
were analysed as a function of stage of sleep (stage 2 sleep,
SWS and REM sleep).

Procedures

General laboratory procedures

During the week before participating in the sleep sessions, subjects were required to maintain their usual bed and rising times on each day. During this period they completed a sleep diary and wore a wrist actigraph (Mini-Logger Series 2000, Mini-Mitter Co. Inc., Sunriver, OR). This was to ensure that they complied with the instructions and to confirm the timing of their normal schedule. All measures were collected using a Grass Model 7D pen-chart recorder (Grass Instrument Co., Quincy, MA) and, with the exception of the submental EMG and the EOG, were stored on a Pentium PC via a 12 bit A/D converter using an acquisition program developed within the laboratory. The rate of digitization was 100 Hz for EEG signals and 2000 Hz for cardiac variables.

Assessment of sleep–wake state

Subject’s sleep–wake state was assessed by standard sleep recordings and procedures (Rechtschaefen and Kales 1968), consisting of central (C3 – A2) and occipital (O1 – A2) EEGs, a submental electromyogram (EMG) and an electro-oculogram (EOG) (left and right outer canthi, offset from the horizontal). Analysis of sleep–wake state was performed by an experienced scorer (JT) by visual analysis of the sleep recordings, although one modification was made to the standard scoring rules. All 30-s epochs containing a sleep disturbance were classified as stage 1, even if they would formally have been identified as stage 2, 3, 4 or REM. This was to ensure that at later stages of data processing epochs of sleep identified for analysis would be artefact free.

Assessment of cardiac variables

The cardiac variables measured were the ECG, cardiac impedance and BP. The dependent variables derived from these measures were HR, the LF and high frequency (HF) bands from spectral analysis of HRV, PEP, and systolic (SBP) and diastolic (DBP) blood pressure.

The ECG was recorded through Meditrace Ag/AgCl spot electrodes. Electrodes were placed on subject’s lower left and lower right rib cage and a third on the right clavícula notch. The right rib cage electrode served as the ground and the remaining two as recording sites. During subsequent analyses R waves were detected using an automated algorithm, allowing IBI to be calculated by the program. The detection of R waves was visually checked and edited where the automatic detection was incorrect.

Cardiac impedance was measured via a Minnesota impedance cardiograph, model 304B (Surcom Inc., Minneapolis, MS) according to standardised procedures (Sherwood et al. 1990). Meditrace Ag/AgCl electrodes (Graphic Controls Corp., Buffalo, NY) were placed 4 cm above the clavicle on the front of the neck and over the sternum at the fourth rib in order to record the signal. The imposed current was applied by electrodes placed over the fourth cervical vertebra on the back of the neck, and on the back over the ninth thoracic vertebra. A 4 mA AC current at 100 kHz was applied through the two stimulating electrodes and basal impedance and rate of change in the impedance waveform on a given beat (dZ/dt) were estimated from the resulting signal.

Pre-ejection period was calculated for each cardiac cycle as the time interval between the Q wave on the ECG signal and the B point on the dZ/dt signal (Sherwood et al. 1990). This was achieved using an automated algorithm. The decisions of the algorithm were visually checked by an experimenter and edited where necessary. Pre-ejection period is sensitive to after load, increasing as a function of the arterial pressure that the contracting ventricle has to work against to open the semilunar valve. Thus, a fall in DBP during sleep would act to decrease PEP, implying a rise in sympathetic activity, and masking a sleep-related decrease in such activity. In view of this, a correction factor for the effect of arterial pressure was determined. This was based on the slope of the linear regression line between the beat to beat DBP and PEP values within each 2-min epoch. It was assumed that variations in BP within 2-min epochs of stable sleep, particularly NREM sleep, would not reflect variations in autonomic balance and would therefore reflect the effect of DBP on PEP. A comparison of the slope values over stages within and between subjects, indicated a high degree of consistency, both between sleep stages and between subjects, with an average value of 0.22 ms mmHg⁻¹. Both the original and corrected values have been reported.

Power spectrum analysis of the HRV data was conducted to determine activity in the HF and LF bands in accordance with procedures outlined in two recent reports (Berntson et al. 1997; Task Force 1996). The IBI time series for each 2-min data epoch selected for analysis (see data analysis section below) was first re-sampled at a frequency of 4 Hz. The time series was then de-trended using a third order polynomial with a 20 s (81 point) window. The effect of this size window was to filter the DC component, but leave intact LF activity. Power spectrum analysis was then applied to the time series. The program calculated the power spectrum density estimate for frequency bins that were then combined to form frequency bands 0.02 Hz wide. Thus, the total power spectrum ranged from 0 to 0.5 Hz in 0.02 Hz bands. To identify the LF component the algorithm searched for the greatest value in the frequency bands from 0.03 to 0.15 Hz. The width of the LF component was defined by the first frequency bands either side of the peak to fall to 50% of the peak value. The area between and including these frequency bands was then integrated. The same procedure was used to identify the HF component, with the exception that the peak value was identified between 0.15 and 0.40 Hz. In accordance with expert recommendations (Task Force 1996) power within the LF and HF bands was quantified using three different methods, each of which has its own advantages and disadvantages. The three methods were: absolute integrated power in arbitrary units; absolute power expressed as standard scores, standardized within a recording session; and power within a band expressed as a proportion of total power.
In addition to these measures, the frequency of the HF peak value was analysed as a measure of respiratory rate (RR) (Brown et al. 1993). The purpose of this analysis was to assess the contribution of changes in RR to any observed change in the HF component of HRV. More intrusive measures of respiration were not conducted to avoid disturbing sleep and influencing autonomic activity.

Blood pressure was measured using a continuous finger blood pressure device (Portapres, Model 2). This apparatus provides continuous assessment of BP. It is also minimally intrusive as it reduces pain associated with cuff inflation by regularly alternating measurements between two fingers. It also provides an automated height adjustment feature. As body movements may move cuffs sufficiently to prevent the automatic calibration from operating appropriately and because inappropriate cuff application can cause baseline shifts between cuffs, each night’s data was specifically inspected for events of this type. However, such difficulties were minimal, and in no instance was a night’s recording lost. Further, because peripheral arterial tone of the finger is minimal, and in no instance was a night’s recording lost. The resulting values were then sorted according to sleep–wake-state (presleep wakefulness and sleep), time (30-min bins) and stage of sleep (stage 2, SWS and REM sleep). Thus, during presleep wakefulness time was divided into four 30-min bins. During the 7 h of sleep it was divided into 14, 30-min bins within each sleep state. Within each cell of the design, where there was more than one 2-min epoch, values were averaged within a night and then nights averaged. Thus, the total matrix consisted of four bins for 14 subjects over the presleep period, and 14 bins by 3 stages for 14 subjects over the sleep period.

Data reduction

The data were analysed in 2-min epochs. An epoch length of 2 min was selected as it is within the recommended range for the LF component (Task Force 1996), and because intervals meeting the criteria set out below become increasingly rare as epoch length increases. The epochs were identified over the 2 h presleep period and during sleep. All possible epochs were selected, according to the following rules:

1 The 2 min before the epoch and the epoch itself had to be: free of body movements; indications of arousal, such as abrupt changes in EEG frequency, bursts of EMG activity, or eye movements; and other artefacts.

2 There could not be a stage change during the 4 min, other than between stages 3 and 4.

3 Once an epoch was identified, another epoch could not be identified for further 5 min, unless there was a change of sleep stage.

4 Epochs were not identified during periods of wakefulness within the sleep period or during stage 1 sleep. This was because in these young subjects, these stages were transitory and contaminated with movement artefact.

Thus, data was analysed as a function of presleep wakefulness, stage 2 sleep, SWS and REM sleep.

Once identified a 2-min epoch was subjected to each of the analysis algorithms to yield beat by beat measures of HR, SBP, DBP and PEP. These were averaged over the 2-min epoch. In addition, single values representing the LF and HF components of HRV were identified. Thus, a single value for each 2-min epoch was calculated for each dependent variable (considering the three different methods of quantifying the LF and HF spectral bands as separate dependent variables).

RESULTS

The nature of the sleep attained by subjects under the recording conditions is shown in Table 1. The distribution of
sleep stages and sleep efficiency values (sleep efficiency of approximately 95%) indicate that the sleep of the subjects was minimally disturbed by the recording procedures and that, as a consequence, the cardiac activity measured in the study can be considered characteristic of normal sleep in young healthy individuals.

The number of subjects for whom data was available differed over variables as a consequence of equipment failure, elimination of data as a result of artefacts and, for BP, because the equipment was not available for four subjects. The N for each of the analyses has been indicated in the relevant Tables.

### Presleep wakefulness vs. sleep

As shown in Table 2, HR and BP were significantly lower in both stage 2 and SWS than presleep wakefulness. Heart rate was also significantly reduced in REM sleep, while BP during REM was approximately at wakefulness levels. The HF component of HRV was higher during NREM sleep than wakefulness, irrespective of the method of quantification. The HF component was also higher during REM sleep as assessed by HFpw, but not when assessed by HFpz or HFpn. While the LF component was reduced in all measures during NREM sleep, the reduction was not significant for LFpw and LFpz for stage 2. LFpw was higher during REM sleep than wakefulness, but not LFpz, while LFpn was significantly lower. PEP, the alternative measure of central sympathetic cardiac activity, was higher in all sleep states (lower sympathetic activity) compared with wakefulness.

Thus, the data was quite consistent during NREM sleep. The three measures of high and low frequency activity gave relatively consistent results, such that the HF component went up and the LF down, although changes in the absolute measures of the LF component (LFpw and LFpz) were small. Further, the two measures of SNS activity were in agreement, showing a reduction in activity. Finally, both HR and BP were lower during NREM sleep. In one respect the data for REM sleep were also consistent, in that REM values were typically more similar to wakefulness than were NREM values. Nevertheless, the results for REM sleep varied more with different measures. For example, some measures suggested that SNS activity was lower in REM sleep than wakefulness (PEP and LFpn), while other measures showed it to be higher (LFpw and LFpz).

### Presleep wakefulness vs. NREM during the first 30 min sleep period bin

This comparison provided a measure of the effect of sleep onset. Both HR and BP were lower during stage 2 sleep as compared with wakefulness, although the effect was relatively small and was not significant for BP (see Table 3). However, during SWS both HR and BP were significantly below the wakefulness level. The different patterns of change over sleep onset are illustrated in Figs 1 and 2 for HR, and SBP and DBP, respectively. The HF component was generally higher for all measures in both stage 2 sleep and SWS. Although the effect only reached statistical significance for HFpw and HFpn in stage 2 and HFpn in SWS, it approached significance for all

### Table 1

<table>
<thead>
<tr>
<th>Sleep stage</th>
<th>TIB (%)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep efficiency</td>
<td>95.08</td>
<td>2.44</td>
</tr>
<tr>
<td>Time awake</td>
<td>4.92</td>
<td>2.44</td>
</tr>
<tr>
<td>Movement time</td>
<td>0.24</td>
<td>0.23</td>
</tr>
<tr>
<td>Stage 1</td>
<td>12.51</td>
<td>4.60</td>
</tr>
<tr>
<td>Stage 2</td>
<td>43.86</td>
<td>7.35</td>
</tr>
<tr>
<td>Stage 3</td>
<td>6.66</td>
<td>2.41</td>
</tr>
<tr>
<td>Stage 4</td>
<td>11.91</td>
<td>4.85</td>
</tr>
<tr>
<td>Stage SWS</td>
<td>18.57</td>
<td>6.47</td>
</tr>
<tr>
<td>Stage REM</td>
<td>19.90</td>
<td>2.46</td>
</tr>
<tr>
<td>Time in bed* (min)</td>
<td>467.4</td>
<td>47.1</td>
</tr>
</tbody>
</table>

*TIB during light out.

LF component was reduced in all measures during NREM sleep, the reduction was not significant for LFpw and LFpz for stage 2. LFpw was higher during REM sleep than wakefulness, but not LFpz, while LFpn was significantly lower. PEP, the alternative measure of central sympathetic cardiac activity, was higher in all sleep states (lower sympathetic activity) compared with wakefulness.

### Table 2

<table>
<thead>
<tr>
<th>d.f.</th>
<th>Pre-sleep</th>
<th>Stage 2</th>
<th>t</th>
<th>All night sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SWS</td>
<td>REM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (beats min⁻¹)</td>
<td>13</td>
<td>64.3 (13.0)</td>
<td>57.2 (10.0)</td>
<td>5.25***</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>9</td>
<td>108.2 (12.6)</td>
<td>97.3 (11.0)</td>
<td>4.84***</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>9</td>
<td>55.6 (7.8)</td>
<td>51.5 (6.5)</td>
<td>3.25*</td>
</tr>
<tr>
<td>HFpw (arbitrary)</td>
<td>12</td>
<td>72.0 (55.6)</td>
<td>185.7 (160.6)</td>
<td>3.21***</td>
</tr>
<tr>
<td>HFpz (z scores)</td>
<td>12</td>
<td>-0.66 (0.74)</td>
<td>0.28 (0.30)</td>
<td>3.78**</td>
</tr>
<tr>
<td>HFpn (prop)</td>
<td>12</td>
<td>0.134 (0.06)</td>
<td>0.222 (0.10)</td>
<td>5.04***</td>
</tr>
<tr>
<td>LFpw (arbitrary)</td>
<td>12</td>
<td>141.2 (104.8)</td>
<td>122.4 (85.5)</td>
<td>1.70</td>
</tr>
<tr>
<td>LFpz (z scores)</td>
<td>12</td>
<td>0.19 (0.59)</td>
<td>-0.07 (0.24)</td>
<td>1.50</td>
</tr>
<tr>
<td>LFpn (prop)</td>
<td>12</td>
<td>0.020 (0.08)</td>
<td>0.145 (0.07)</td>
<td>5.68***</td>
</tr>
<tr>
<td>PEP (ms)</td>
<td>13</td>
<td>100.1 (9.45)</td>
<td>107.3 (9.54)</td>
<td>3.65***</td>
</tr>
<tr>
<td>PEP ms (corrected)</td>
<td>9</td>
<td>87.3 (10.3)</td>
<td>96.5 (10.2)</td>
<td>4.98***</td>
</tr>
<tr>
<td>HF0 (Hz)</td>
<td>12</td>
<td>0.287 (.045)</td>
<td>0.255 (.038)</td>
<td>3.45***</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, ***P < 0.001. See text for abbreviations.
other comparisons. The e/C128ect is illustrated in Fig. 3. The LF component fell at sleep onset, being significantly lower during SWS for all measures (see Table 3 and Fig. 4), although only LFpn was significant for stage 2. Further, SWS values in the first 30-min bin approximated the total sleep period SWS values. In contrast, PEP did not change significantly from presleep wakefulness to the first sleep period bin for either stage 2 sleep or SWS. Thus, with the exception of PEP, the sleep related changes identified in the previous section were established relatively rapidly, being evident during the first 30-min bin following lights out.

It is of interest to note that there was a non-significant fall in PEP of approximately 4 ms during SWS. As it seems unlikely that SNS activity had indeed increased over the sleep onset period, this effect seems most likely to be caused by the fall in BP. Consistent with this, corrected PEP showed no change over the sleep onset period.

### Table 3
Mean values for presleep wakefulness and the first 30 min sleep bin for stage 2 and SWS ($t$-values compare wakefulness with each sleep stage. SD in brackets.)

<table>
<thead>
<tr>
<th></th>
<th>Stage 2</th>
<th></th>
<th>SWS</th>
<th></th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.f.</td>
<td>Wake</td>
<td>Stage 2</td>
<td></td>
<td>t</td>
</tr>
<tr>
<td>HR (beats min$^{-1}$)</td>
<td>13</td>
<td>64.3 (13.0)</td>
<td>59.8 (11.4)</td>
<td>4.57***</td>
<td>12</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>9</td>
<td>108.2 (12.6)</td>
<td>104.0 (12.3)</td>
<td>1.39</td>
<td>8</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>9</td>
<td>55.6 (7.8)</td>
<td>55.1 (12.5)</td>
<td>0.13</td>
<td>8</td>
</tr>
<tr>
<td>HFpw (arbitrary)</td>
<td>12</td>
<td>72.0 (55.6)</td>
<td>158.6 (163.3)</td>
<td>2.51*</td>
<td>12</td>
</tr>
<tr>
<td>HFpz (z scores)</td>
<td>12</td>
<td>-0.66 (0.74)</td>
<td>-0.03 (0.90)</td>
<td>2.09</td>
<td>10</td>
</tr>
<tr>
<td>HFpn (prop)</td>
<td>12</td>
<td>0.134 (0.06)</td>
<td>0.195 (0.09)</td>
<td>2.93*</td>
<td>11</td>
</tr>
<tr>
<td>LFpw (arbitrary)</td>
<td>12</td>
<td>90.5 (9.74)</td>
<td>99.3 (9.33)</td>
<td>0.52</td>
<td>12</td>
</tr>
<tr>
<td>LFpz (z scores)</td>
<td>12</td>
<td>0.220 (0.08)</td>
<td>0.156 (0.08)</td>
<td>3.01*</td>
<td>11</td>
</tr>
<tr>
<td>LFpn (prop)</td>
<td>12</td>
<td>0.027 (0.045)</td>
<td>0.265 (0.045)</td>
<td>2.79*</td>
<td>10</td>
</tr>
</tbody>
</table>

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. See text for abbreviations.

### Stage and time during the sleep period
The results of the statistical analyses for HR during the sleep period are reported in Table 4 and the data illustrated in Fig. 1. Heart rate was higher during REM sleep, but did not differ between stage 2 and SWS. In addition to the fall at sleep onset during SWS, the LF component fell at sleep onset, being significantly lower during SWS for all measures (see Table 3 and Fig. 4), although only LFpn was significant for stage 2. Further, SWS values in the first 30-min bin approximated the total sleep period SWS values. In contrast, PEP did not change significantly from presleep wakefulness to the first sleep period bin for either stage 2 sleep or SWS. Thus, with the exception of PEP, the sleep related changes identified in the previous section were established relatively rapidly, being evident during the first 30-min bin following lights out.

It is of interest to note that there was a non-significant fall in PEP of approximately 4 ms during SWS. As it seems unlikely that SNS activity had indeed increased over the sleep onset period, this effect seems most likely to be caused by the fall in BP. Consistent with this, corrected PEP showed no change over the sleep onset period.

### Figure 1
Mean heart rate (HR) during presleep wakefulness and sleep as a function of time (30 min bins) and stage of sleep. SO indicates sleep onset.

### Figure 2
Mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) during presleep wakefulness and sleep as a function of time (30 min bins) and stage of sleep. SO indicates sleep onset.

onset, HR fell during much of the sleep period, with an asymptote towards morning. The effect of time was significant in all analyses and, although there was some variability in SWS, as indicated by a significant interaction effect for this stage, the effect of time was reasonably consistent over stages.

The effect of stage of sleep on BP was similar to the effect on HR. Both SBP and DBP were higher during REM sleep than NREM sleep throughout the sleep period, while stage 2 did not differ from SWS. However, the changes over time were different. Blood pressure, like HR, fell rapidly at the onset of sleep, but unlike HR, did not continue to fall during the sleep period. Rather, if anything, there was a tendency for BP to gradually increase, although the effect of time was only significant for 1 of the 3 DBP analyses (see Table 4 and Fig. 2). Of interest were the changes in BP during the first hour or so of sleep (see Fig. 2 and Table 3). Blood pressure fell rapidly in SWS, but more gradually in stage 2, with stage 2-values not reaching SWS levels until the third half-hour bin.

Analyses of the HF component conducted within the sleep period showed that there was a trend for HF activity to be higher in the NREM stages than REM. This was significant for stage 2 vs. REM for all methods and was significant for SWS compared with REM for HFpn (see Table 4). SWS and stage 2 were not different, except for HFpn. Further, there was not a marked effect of time, although stage 2 and REM showed a very slight tendency to be higher in the middle of the night. (see Table 4 and Fig. 3). Thus, HF activity rose sharply at sleep onset and remained elevated at a relatively constant level throughout the sleep period, with a general tendency to be higher in NREM than REM sleep.

Table 4  F ratios for the analyses of the effect of stage of sleep and time of night during the sleep period

<table>
<thead>
<tr>
<th>Variable</th>
<th>Comparison</th>
<th>Stage</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Time</td>
<td>Stage × Time</td>
</tr>
<tr>
<td>HR</td>
<td>Stage 2 vs. SWS</td>
<td>11,13 = 0.17</td>
<td>9,117 = 6.18***</td>
</tr>
<tr>
<td>n = 14</td>
<td>Stage 2 vs. REM</td>
<td>11,13 = 22.12***</td>
<td>11,143 = 15.19***</td>
</tr>
<tr>
<td>SWS vs. REM</td>
<td>11,13 = 8.18*</td>
<td>7,91 = 2.92**</td>
<td>7,91 = 1.18</td>
</tr>
<tr>
<td>SBN</td>
<td>Stage 2 vs. SWS</td>
<td>11,13 = 0.54</td>
<td>8,91 = 1.50</td>
</tr>
<tr>
<td>n = 10</td>
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<td>11,99 = 0.88</td>
</tr>
<tr>
<td>SWS vs. REM</td>
<td>11,13 = 15.84***</td>
<td>7,63 = 0.43</td>
<td>7,63 = 1.32</td>
</tr>
<tr>
<td>DBP</td>
<td>Stage 2 vs. SWS</td>
<td>11,13 = 0.32</td>
<td>8,91 = 1.39</td>
</tr>
<tr>
<td>n = 10</td>
<td>Stage 2 vs. REM</td>
<td>11,13 = 21.53***</td>
<td>11,99 = 2.37*</td>
</tr>
<tr>
<td>SWS vs. REM</td>
<td>11,13 = 8.05*</td>
<td>7,63 = 2.01</td>
<td>7,63 = 1.72</td>
</tr>
<tr>
<td>HFpn</td>
<td>Stage 2 vs. SWS</td>
<td>11,13 = 0.23</td>
<td>8,91 = 1.83</td>
</tr>
<tr>
<td>n = 13</td>
<td>Stage 2 vs. REM</td>
<td>11,13 = 7.91*</td>
<td>11,132 = 2.19*</td>
</tr>
<tr>
<td>SWS vs. REM</td>
<td>11,13 = 3.68</td>
<td>7,63 = 1.25</td>
<td>7,63 = 0.98</td>
</tr>
<tr>
<td>HFpn</td>
<td>Stage 2 vs. SWS</td>
<td>11,13 = 0.00</td>
<td>8,91 = 1.11</td>
</tr>
<tr>
<td>n = 13</td>
<td>Stage 2 vs. REM</td>
<td>11,13 = 11.04***</td>
<td>11,132 = 3.49***</td>
</tr>
<tr>
<td>SWS vs. REM</td>
<td>11,13 = 2.10</td>
<td>7,63 = 1.65</td>
<td>7,63 = 0.45</td>
</tr>
<tr>
<td>HFpn</td>
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<td>8,91 = 1.37</td>
</tr>
<tr>
<td>n = 13</td>
<td>Stage 2 vs. REM</td>
<td>11,13 = 35.36***</td>
<td>11,132 = 1.93*</td>
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<tr>
<td>SWS vs. REM</td>
<td>11,13 = 31.46***</td>
<td>7,63 = 1.61</td>
<td>7,63 = 0.64</td>
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</tr>
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<td>11,132 = 0.75</td>
</tr>
<tr>
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<td>7,63 = 0.86</td>
<td>7,63 = 0.74</td>
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<td>8,91 = 0.63</td>
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<td>11,13 = 17.49***</td>
<td>11,132 = 0.60</td>
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<td>SWS vs. REM</td>
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<td>7,63 = 1.08</td>
<td>7,63 = 0.29</td>
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<td>8,91 = 1.40</td>
</tr>
<tr>
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<td>11,13 = 36.04***</td>
<td>11,132 = 0.97</td>
</tr>
<tr>
<td>SWS vs. REM</td>
<td>11,13 = 16.30**</td>
<td>7,63 = 1.07</td>
<td>7,63 = 1.05</td>
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<td>9,117 = 10.93***</td>
</tr>
<tr>
<td>n = 14</td>
<td>Stage 2 vs. REM</td>
<td>11,13 = 9.48***</td>
<td>11,143 = 6.42***</td>
</tr>
<tr>
<td>SWS vs. REM</td>
<td>11,13 = 7.75*</td>
<td>7,91 = 1.92</td>
<td>7,91 = 0.44</td>
</tr>
<tr>
<td>PEPcor</td>
<td>Stage 2 vs. SWS</td>
<td>11,13 = 1.68</td>
<td>8,91 = 11.60***</td>
</tr>
<tr>
<td>n = 10</td>
<td>Stage 2 vs. REM</td>
<td>11,13 = 13.03***</td>
<td>11,99 = 2.33*</td>
</tr>
<tr>
<td>SWS vs. REM</td>
<td>11,13 = 3.28</td>
<td>7,63 = 4.26**</td>
<td>7,63 = 1.49</td>
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<td>Stage 2 vs. SWS</td>
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<td>8,91 = 2.58**</td>
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<tr>
<td>n = 13</td>
<td>Stage 2 vs. REM</td>
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<td>11,132 = 1.20</td>
</tr>
<tr>
<td>SWS vs. REM</td>
<td>11,13 = 11.39**</td>
<td>7,63 = 0.43</td>
<td>7,63 = 0.28</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, ***P < 0.001. See text for abbreviations.
Low frequency power was significantly higher during REM sleep than either of the NREM sleep stages for all three measures. Further, unlike the other variables SWS values tended to differ from stage 2, with SWS being lower. This comparison was significant for LFpw and LFpz. The effects were consistent over the night with non-significant effects of time, for all analyses and only one marginally significant interaction effect (Table 4 and Fig. 4). However, PEP showed a different pattern (Table 4 and Fig. 5). Unlike all other variables, there was no significant change at the onset of sleep. Rather, PEP increased progressively during the sleep period, in all sleep stages (decrease in sympathetic activation), with little difference between the stages.

**DISCUSSION**

With respect to the effect of sleep stage, the data is broadly consistent with the major trends in the literature as to the effect of NREM sleep on cardiac activity and on measures that reflect autonomic control of cardiac activity. In particular, HR and BP fell during NREM sleep and there was an increase in the HF component of HRV and a decrease in the LF component, particularly when the LF component was expressed as a proportion of total variability. Most importantly, the investigation of the relationship between stage of sleep and time revealed several novel observations. First, BP and the LF and HF components of HRV were not affected by time during the sleep period, suggesting that time of night effects are because of the changing distribution of sleep stages.
In contrast to measures of systolic timing, HR and PEP, changed over time within each sleep stage. Second, most measures, but BP and the HF component of HRV in particular, showed abrupt and substantial changes at sleep onset. Third, there were few differences between stage 2 and SWS, suggesting degree of cortical desynchronisation has little effect on cardiac activity during sleep. Finally, the data suggested that the frequently reported finding that the LF component of HRV is lower during NREM sleep than wakefulness, may in part depend on the data being expressed as a proportion of total power, an effect that may primarily reflect changes in the HF component.

In contrast to some reports in the literature there were few differences between stage 2 and SWS (HF\textsubscript{pa} and the LF component expressed as absolute power, were the exceptions). For those comparisons that were significant the differences were relatively small and were typically most prominent during and immediately following sleep onset. Thus, for all variables except PEP, SWS showed a larger sleep effect than stage 2 during the first 30-min bin (see Table 3). This pattern suggests that the differences between stage 2 and SWS were not stage related, but rather occurred as a consequence of the time course of variables attaining their NREM sleep levels. The relative absence of differences over the total sleep period indicates cardiac activity is not strongly influenced by the ‘depth of NREM sleep’ or by the physiological processes associated with cortical synchronization. Rather, from a cardiac perspective, NREM sleep appears to consist of a uniform state. The results may also explain current discrepancies in the literature with respect to differences between stage 2 and SWS. According to the present data, a difference would be expected if the data analysed came from the first sleep cycle, but not if data were obtained throughout sleep or at some other time during sleep.

While cardiac activity differed little between stage 2 and SWS, there were consistent and marked differences between the NREM stages and REM sleep. Heart rate, BP and the LF component were all higher in REM, while the HF component was lower. Only PEP failed to show a REM–NREM difference. However, the relationship between REM sleep and wakefulness was in some respects less consistent. In terms of functional change, some variables suggested cardiac activity was higher in REM, for example, absolute LF power, while others suggested it was lower, for example, absolute HF power, while a number showed no difference, for example BP. Further, there were inconsistencies over different methods of quantifying HRV. On the other hand, the results for REM sleep did show one relatively consistent feature in that values for REM sleep tended to be closer to wakefulness values (whether above or below wakefulness) than NREM values.

Unexpectedly, with the exception of HR and PEP, cardiac activity and cardiac control were shown to be relatively independent of time within the sleep period. The absence of time of night effects for BP and the LF and HF components of HRV suggests that the level of these variables during the sleep period are a function of sleep and do not reflect either time asleep or circadian system influences. The absence of a circadian influence is consistent with previous work on BP (Kerkhof et al. 1998) and the LF component of HRV (Burgess et al. 1997). However, the present data are inconsistent with a previous report of a circadian influence over the HF component of HRV (Burgess et al. 1997), although it should be noted
that the earlier study was run using a constant routine procedure, and thus may have been more sensitive to circadian influences.

Heart rate showed significant reductions at sleep onset and continued to fall throughout sleep, a pattern consistent with a combined sleep and circadian influence over this variable. This interpretation is supported by previous studies that have used constant routine methodology (Burgess et al. 1997; Kerkhof et al. 1998). The other variable to show a significant effect of time was PEP, a result consistent with previous analyses of this variable (Burgess et al. 1997, 1999). However, this may not be interpretable as a circadian effect as it has been shown that PEP does not change over the sleep period in the absence of sleep (Burgess et al. 1997). At this time therefore it is more interpretable as an effect of time asleep.

A number of studies have suggested that cardiac activity shows systematic changes over the sleep period (Degaute et al. 1991; Furlan et al. 1990; Parati et al. 1990; van de Borne et al. 1994). The present results indicate that BP and the frequency components of HRV do not change significantly over time within either NREM or REM sleep. As these variables do differ markedly between NREM and REM, the results suggest changes observed over the sleep period are because of a change in the distribution of NREM and REM sleep. However, the change in HR over the sleep period reflected both NREM–REM distribution and changes within stages, the latter reflecting a circadian input. In contrast, as noted above, the increase in PEP over the sleep period appears to be a direct effect of time asleep.

It is of interest to note that most variables changed rapidly at sleep onset, attaining their NREM values within the first half-hour after lights out. This is consistent with the concept that the changes are actively induced, rather than being a passive consequence of being asleep. The pattern of change in BP at sleep onset was of particular interest. Consistent with one previous report (Degaute et al. 1991), the fall was shown to be very abrupt. However, the rate of fall was stage dependent. Thus it was extremely rapid in SWS, but taking longer to reach minimum values in stage 2. This does not appear to be a direct stage effect as later in the sleep period BP did not differ between stage 2 and SWS. A possible explanation is that it is related to arousal from sleep. Thus, early in sleep in young adults the pressure for SWS is so intense that stage 2 sleep only occurs immediately after an arousal from sleep. The effect of such an arousal on BP might be large enough for it to affect stage 2 epochs, despite requiring 2 min of undisturbed sleep before a 2-min epoch was included in the analysis. This would not be an issue later in sleep as here there are extended periods of stage 2 sleep.

A major issue in the investigation of cardiovascular activity during sleep in humans is the interpretation of the measures available to assess autonomic nervous system activity. Two methods were used in the present study, PEP and the high and low frequency components of HRV. The concern with PEP in sleep studies is its susceptibility to influence by after load. In addition, there are a number of methodological considerations with respect to the high and low frequency components of HRV. The first is the purity with which the LF component reflects SNS activity (noting that there is wide acceptance that the HF component reflects PNS activity). In light of this PEP was analysed as an alternative measure of SNS activity. The second concern is the method used to quantify the components. Thus, in accord with recommendations (Task Force 1996), three methods of quantification were applied to the data. The third difficulty is that the HF component can vary as a function of respiratory activity, independent of vagal output.

A decrease in central sympathetic activity results in an increase in PEP as a consequence of a decrease in the innervation of the left ventricle. However, a decrease in arterial BP (decreased after load) causes a decrease in PEP, as the load the contraction has to work against is reduced. Thus the fall in BP observed during sleep would tend to decrease PEP. However, in this study, PEP increased during sleep. Further, while PEP increased progressively over the sleep period, BP did not change appreciably. Thus, the pattern of change over the sleep period was unlikely to be an artefact of BP, although the magnitude of the decrease in SNS activity may have been underestimated because of masking by BP. However, certain features of the data for PEP may have been a consequence of after load. Thus, the paradoxical, although not significant, fall in PEP (increase in SNS activity) during the initial SWS period may have been because of the rapid fall in BP at sleep onset. Similarly, the failure to obtain a NREM–REM difference in PEP may have been a consequence of the BP difference between NREM–REM sleep. Of interest was the observation that the after load correction factor eliminated the fall in PEP at sleep onset, but did not affect the rise in PEP over the sleep period or the relationship between NREM and REM sleep. This suggests these latter two findings may genuinely reflect sympathetic innervation of the left ventricle. One possibility is that the increase in PEP reflects the same control system as HR, noting their physiological interdependence (Sherwood et al. 1990) and the similarity of their time course over the sleep period in the current data.

There were points of agreement and points of discrepancy between the two measures of SNS activity. Most importantly, both indicated a reduction in SNS activity during sleep. This result offers support for the view that the LF component of HRV reflects SNS activity. However, there was reasonable evidence to suggest that the pattern of change over the sleep period was different for the two measures, both in terms of changes at sleep onset and in changes as sleep progressed. The status of the difference with respect to sleep stage is less certain and the absence of a difference in PEP between NREM and REM sleep may reflect the effect of after load. It remains unclear whether the LF component reflects SNS activity because it provides an index of cardiac sympathetic activation (Malliani et al. 1994; Pagani et al. 1986), or indirectly via baroreflex-mediated cardiac vagal responses to arterial BP fluctuations of sympathetic vasomotor origin (Berntson et al. 1996). Finally, it should also be acknowledged that BP assessed at the periphery may not be appropriate as an
estimate of after load and thus the particular pattern of change in PEP may more reflect methodological limitations, rather than a particular pattern of autonomic change.

The three methods of quantifying the components of HRV showed relatively consistent results. In particular, the two representations of absolute power showed good agreement, indicating that with reasonably sized data sets the variation in total power is not a critical problem. However, there were also important differences that appeared to be due to the interdependency of the two components when power within a frequency band was expressed as a proportion of total power. First, the methods produced variable results for REM sleep. Thus, the proportional measure exaggerated differences between wakefulness and NREM sleep, because the HF and LF effects complemented each other. On the other hand there were more complex consequences for REM sleep, because the HF and LF effects tended to be in opposition. Second, proportional representation of LF power considerably exaggerated the difference between wakefulness and NREM sleep as a consequence of an indirect effect of the HF component. Thus, representation of data as proportional power to eliminate the problem of individual differences in absolute power, may do more to conceal effects, than to illuminate them.

The HF component of HRV is modulated by two aspects of respiratory activity. Power is negatively associated with RR and positively associated with tidal volume (Berntson et al. 1996). As sleep onset is associated with a reduction in RR (Phillipson and Bowes 1986; Trinder et al. 1992), the rapid rise in the HF component with sleep onset could be an artefact of the fall in RR. However, in contrast, the fall in tidal volume that also occurs at this time (Phillipson and Bowes 1986; Trinder et al. 1992) would tend to oppose any rise in the HF component.

In view of these considerations we analysed HFfr as a measure of the effect of sleep on RR. More invasive measures were not employed in order to minimize disruption of sleep and autonomic activity. Consistent with previous literature, HFfr indicated RR was lower during sleep than wakefulness, with the timing of the fall coinciding with the rise in the HF component. While this pattern was consistent with the view that the sleep effect on the HF component is an artefact of a change in RR, the relationship amongst the sleep stages was not. If the stage differences in HF activity were a consequence of RR it would have been expected that RR would have been higher in REM sleep than NREM, not lower. Further, the smallest sleep effect was in SWS, yet at sleep onset the HF component tended to be larger in SWS. Thus, it appears unlikely that the elevated HF activity during NREM sleep was an artefact of low RR. Indeed, this is not surprising as the mean frequency of the peak HF value only varied between 0.24 and 0.29 Hz. In this region the relationship between RR and the HF component is essentially flat (Brown et al. 1993). Thus, in sleep studies the intensity of respiratory activity does not appear to be a major confound in the interpretation of the HF component. This conclusion is consistent with previous analyses of this issue (e.g. Burgess et al. 1997).

While considerable effort has been extended to address some of the methodological issues surrounding HRV analysis it must be stressed that the LF and HF components are a complex interaction between neural input and sinus node responsiveness. For example, recent data has suggested that the interaction between the two components is not linear (Rocchetti et al. 2000), further complicating interpretation.

The present results confirm and extend previous studies of cardiac activity and autonomic control of cardiac activity during sleep. The data indicate that PNS activity increases with the onset of sleep and remains at a high level throughout NREM sleep, an effect that does not appear to be an artefact of sleep-related changes in respiratory activity. During REM sleep PNS activity returns towards wakefulness values, but remains slightly higher. Similarly, SNS activity, as indicated by the LF component of HRV, falls during NREM sleep, an effect that is more marked when proportional representation of the component is used. During REM sleep SNS activity increases above wakefulness levels. Of interest was the observation that neither component of HRV varied as a function of time within a state. Thus, time-of-night effects are likely to be the result of changes in sleep stage distribution. In addition, the analysis of stage of sleep as a function of time indicated that autonomic balance was independent of ‘depth’ of NREM sleep. In effect, the data suggests autonomic balance varies as a square wave between wakefulness and NREM and REM sleep, showing relative sympathetic dominance during wakefulness and REM sleep and relative parasympathetic dominance during NREM sleep.

Pre-ejection period, an alternative measure of SNS activity also indicated a fall in SNS output during NREM sleep. This result, in part, validated the LF component of HRV as a measure of sympathetic activity. However, the pattern of change differed from that of the LF component. While the influence of BP on PEP may have been responsible for some of these differences, for reasons discussed above, it seems unlikely that it would account for all differences. Thus it remains possible there is a degree of fractionation of central cardiac SNS control during sleep.

Finally, the variations in HR and BP are clearly, in part, a consequence of modulations in sympathetic and parasympathetic activity, as a consequence of the influence of sleep. However, HR, at least, is independently determined by the circadian system, and it remains likely that BP also has other determinants.

ACKNOWLEDGEMENTS
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REFERENCES
Berlad, I., Shlitner, S., Ben-Haim, S. and Lavie, P. Power spectrum analysis of heart rate variability in stage 4 and REM sleep: evidence


