CHANGES IN CARDIOVASCULAR FUNCTION DURING THE SLEEP ONSET

PERIOD IN YOUNG ADULTS

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ABSTRACT

Blood pressure (BP) and heart rate (HR) are influenced by the sleep-wake cycle, with relatively abrupt falls occurring in association with sleep onset (SO). However, the pattern and rate of fall in BP and HR during SO, and the processes that contribute to the fall in these variables have not been fully identified. Continuous BP and HR recordings were collected beginning 1 h before lights out (LO) until the end of the first NREM sleep period in 21 young, healthy participants maintained in a supine position. Five consecutive phases were defined: 1) the 30 mins of wakefulness before LO; 2) LO to stage 1 sleep; 3) stage 1 to stage 2 sleep; 4) stage 2 sleep to the last micro-arousal before stable sleep and; 5) the first 30 minutes of undisturbed stable sleep. The data were analysed on a beat-by-beat basis and reported as 2 min periods for phases 1 and 5, and 10% epochs for phases 2, 3 and 4 (as participants had variable time periods in these phases). The level of baroreflex (BR) activity was assessed by the sequence technique and an autoregressive multivariate model. Further, during phases 3 and 4, the BP and HR responses to arousal from sleep were determined. There were substantial falls in BP and HR following LO before the initial onset of theta activity (phase 3), and again following the onset of stable sleep after the cessation of spontaneous arousals. During phases 3 and 4 when there were repeated arousals from sleep, the fall in both variables was retarded. Further, both the rate and magnitude of the fall in BP were negatively associated with the number of arousals during phases 3 and 4. There was a small increase in the sensitivity of the BR and indirect evidence of a substantial fall in its set point.

Keywords: Blood pressure, heart rate, arousal, baroreflex

INTRODUCTION

Blood pressure (BP) and heart rate (HR) fluctuate over 24 h, being lower during night-time than daytime (4, 14, 20, 22, 25, 50). BP and HR are lower throughout non-rapid eye movement (NREM) sleep than wakefulness, particularly during slow wave sleep, while in rapid eye movement sleep they approximate wakefulness levels (10, 37, 43, 48-50). Constant routine studies that do not allow sleep fail to show a 24 h variation in BP (16, 53), whereas 24 h continuous bed restriction studies that permit sleep, whilst controlling for postural effects, do show a diurnal rhythm (2, 23, 50, 52). Thus, BP changes are due to specific sleep effects superimposed on reductions in activity and postural adjustments (14, 34), yet endogenous circadian factors do not appear to regulate BP. The sleep effect on BP is initiated around the time of sleep onset (SO) and the nocturnal nadir occurs within the first sleep cycle soon after stable sleep is attained, while the remaining sleep period is associated with a gradual rise towards wakefulness levels (8, 14, 48, 50). Alternatively, circadian factors, in addition to the rest-activity cycle, have been implicated in the rhythmicity of HR (7, 16, 18, 53). HR begins to fall earlier in the evening in anticipation of sleep, followed by an increased rate of fall at SO, and reaching a nocturnal nadir several hours later (8, 14, 48, 50).

Thus, the SO period may be more critical for BP than HR; delays in SO lead to delays in the night-time fall in BP, but do not affect the timing of the fall in HR (8). The few studies specifically conducted over the SO period that take account of the interval between wakefulness and stable sleep suggest that the sleep related reductions in BP (34) and HR (6, 35) occur in close association with electroencephalographic (EEG) indications of sleep. However, there is evidence that sleep preparatory behaviour, such as turning off the lights and the cognitive decision to go to sleep, may also influence BP changes in the time immediately preceding EEG evidence of sleep, when participants are still awake (10, 14, 37). Thus, the precise role of sleep *per se*, as distinct from sleep "initiation" factors, in bringing about the falls in BP and HR at SO is ambiguous. Further, it remains unclear whether cardiovascular activity is influenced by a critical physiological "switch" event indexed by alpha–theta EEG changes, as observed in the respiratory system (11, 15, 55). Such an influence would predict that periodic reappearance of EEG alpha activity, indicative of arousal from sleep and accompanied by substantial transient elevations that return BP and HR to approximately pre-sleep wakefulness levels

(6, 13, 26), would impede the sleep related decreases in these variables. However, while the morphology of arousals from sleep has been extensively studied, their effect on the trajectory of the falls in BP and HR in the transition from wakefulness to sleep has not been investigated.

There is evidence to indicate that in severe hypertension and obstructive sleep apnea (OSA), the nocturnal fall in BP is absent, thus giving rise to a "non-dipping" BP profile (27, 28, 44). These patients may be at higher risk for cerebrovascular and cardiovascular complications (28, 29, 54). It is thought that the sleep fragmentation linked to OSA contributes to the non-dipping BP phenomenon as a consequence of the repetitive increases in BP that frequent arousals from sleep induce (13, 26, 46). However, little is known of the effects of repetitive arousals on tonic BP during sleep in normal individuals. In this study, we assessed the effect of the spontaneous arousals that occur in normal individuals during the SO period, on the normal sleep related fall in BP.

Thus, the normal pattern of change in BP, HR and baroreflex (BR) activity, beginning with relaxed pre-sleep wakefulness through the SO period to stable NREM sleep was assessed. The strategy followed was to divide the SO period into a number of phases derived from conventional indications of the progression of SO, in order to provide a description of the impact of EEG changes on cardiovascular function. We hypothesised that the sleep related falls in BP and HR would not occur until arousal from sleep ceased. Thus, it was predicted that the timing of the fall in BP and HR would be such that they occurred with the onset of stable sleep.

METHODS

Participants

The sample comprised ten male and eleven female participants with a mean age of 21.1 ± 2.8 yrs (range 18-26 yrs). Their average BMI was 22.9 ± 2.1 kg/m^2 (range 19.3–26.9 1 kg/m^2). All participants were healthy non-smokers who had not undertaken any shiftwork or trans-meridian travel in the three months prior to participation in the study. The participants were free of physical illness and were not, and had not been taking any medication. Female volunteers were not selected if they were taking a hormonal contraceptive, although menstrual phase was not controlled for. Participants did not engage in intense, regular physical exercise or recreational drug consumption, and did not

consume large amounts of caffeine (>350 mg/day) or alcohol (>5 standard drinks/wk). All participants reported no known personal or family history of a sleep, cardiovascular or respiratory disorder. Further, their sleep records were scrutinised for evidence of any sleep disordered breathing. Lastly, participants were not run in the study during times of major life stress, such as during examination periods. The above data were collected by questionnaire at an intake interview. The project was approved by the Human Research Ethics Committee of The University of Melbourne. Further, participants gave informed consent to participate in the study and were reimbursed for their time commitment to the study.

Design

The study was conducted over one adaptation night and either one (three participants), two (nine participants), or three (nine participants) experimental nights. The aim was to obtain three nights of data from all participants, however the number of nights varied due to participant availability, technical difficulties, and participants being unable to sleep. From each experimental night, the recording period was divided into five phases based on procedural and electrophysiological factors. The phases were derived from conventional beliefs about the normal progression from wake to sleep and resembled criteria described previously (15, 47, 55). The electrophysiological identification of the phases was performed by an experienced scorer by visual analysis of paper chart recordings. Phase 1 consisted of a 30 min period of relaxed wakefulness prior to lights out (LO). At the beginning of this phase, participants had already been in bed in a supine position for a minimum of 30 min. Phase 2 consisted of relaxed wakefulness after LO, before the first occurrence of 10 sec of continuous theta activity. Phase 3 was the time between the first 10 sec of continuous theta activity and the first sleep spindle or K-complex. Phase 3 incorporated alternating periods of alpha and theta activity and was thus synonymous with stage 1 sleep with the continued presence of arousals. Phase 4 corresponded to the time between the first occurrence of a sleep spindle or K-complex and the commencement of stable stage 2 sleep (as defined by at least 5 min of continuous theta activity following a sleep spindle or K-complex). Phase 4 included periods of stage 1 and stage 2 sleep with the continued presence of arousals. Finally, phase 5 consisted of 30 min of NREM sleep, beginning with the onset of stage 2

sleep following the last arousal in phase 4. This phase was characterised by continuous stage 2 or slow wave sleep and by the absence of transient arousals.

Cardiac activity was analysed as a function of the five phases within the SO period. The dependent variables were systolic blood pressure (SBP), diastolic blood pressure (DBP), HR and estimates of arterial BR activity.

During phases 3 and 4 which were characterised by sleep-wake state instability, arousals from sleep were identified in order to assess their effect on cardiovascular activity. Arousals can vary from relatively brief electromyographic (EMG) increases (eliciting non-cortical activation) to increases in cortical EEG desynchronised activation, as shown by sleep stage shifts or full awakenings, with concomitant cardiovascular alterations (40, 41). In recognition of the concept of a hierarchy of arousal responses, the intensity of spontaneous arousals were quantified from EEG, EMG and electrooculographic (EOG) activity. Thus, similar to Davies *et al.* (13), spontaneous arousals were assigned a level of intensity ranging from 1 to 3, with lower grade arousals representing subtle/micro-arousals, level 2 arousals meeting the American Sleep Disorders Association (ASDA) criteria for arousal scoring (1), and level 3 arousals incorporating a motor component (see Table 1 for definitions).

Identification of spontaneous arousals was performed individually by two scorers via visual analysis of the paper chart recordings. If there were discrepancies in arousal classifications between these two scorers, the data were adjudicated by a third person. An intervening sleep period of at least 20 sec of theta activity was required before a new arousal was scored. Thus, re-arousals within this time were regarded as continuous with the previous arousal. The magnitude of SBP, DBP and HR responses to arousal were then analysed as a function of phases 3 or 4 and the three arousal intensities.

[Insert Table 1]

Procedure

General laboratory procedures

Participants' normal sleep-wake patterns were identified by a questionnaire and in the week before and for the duration of their participation in the study, they were required to maintain this pattern. In the 24 h prior to each test session, participants refrained from alcohol and caffeine consumption. An adaptation night preceded the experimental nights. For each session, participants were put to bed at least 1 h before their usual SO time. Throughout this pre-sleep wakefulness period, participants remained in a supine position with their head slightly elevated. They were permitted to read, listen to light music or watch television or a video. Both ambient light and room temperature were kept constant at approximately 50 lux and 22-24 ºC, respectively. The participant's recordings were monitored to ensure they did not go to sleep. At the end of this wake phase at their normal SO time, the lights were turned off and participants were told they could go to sleep. Thereafter, they were left undisturbed until their normal morning awakening time (ten participants) or until their first sleep cycle was completed (eleven participants).

Assessment of sleep-wake state

Participants' sleep-wake state was assessed according to standardised sleep recordings and procedures (36). They consisted of a central (C_3–A_2) and occipital (O_1–A_2) EEG, a submental EMG and an EOG (left and right outer canthi offset from horizontal).

Assessment of heart rate

Participants' HR was assessed with an electrocardiogram (ECG) measured through three Meditrace Ag/AgCl spot electrodes (Graphic Controls Corp., Buffalo, NY, USA). Electrodes were placed on participants' lower left and lower right rib cage and another on the right clavicular notch. The right rib cage electrode served as the ground and the remaining two as recording sites. Analysis of the ECG involved detection of the R-wave of each cardiac cycle using an automated algorithm, allowing inter-beat interval to be calculated by the program. The detection of R-waves was checked visually and edited where the automatic detection was incorrect.

Assessment of blood pressure

BP was assessed by the arterial volume-clamp method (33) providing a continuous non-invasive estimation using a Portapres Model-2 device (TNO-TPD Biomedical Instrumentation, Amsterdam, The Netherlands). Inflatable cuffs were firmly placed around the index and middle fingers and were automatically inflated alternately at 15 min time intervals. Thus, the apparatus was minimally intrusive in that it reduced ischaemic discomfort associated with continuous cuff inflation by regularly alternating measurements between two fingers. The cuffed fingers were maintained at heart level throughout the recording procedure, except for a small number of participants who read during the pre-sleep wakefulness period. This was not considered critical as the equipment incorporated an automated height adjustment.

The BP data were inspected for body movement artefact that may have shifted the cuffs, thus preventing BP from being measured accurately, and for inappropriate cuff application that can cause baseline differences in BP values between the two cuffs. In such circumstances, BP data were not retained, however there were few events of this type and in no instance was a full nights recording lost. Additionally, because peripheral arterial tone of the finger is affected by temperature, the laboratory temperature was set at 22-24 ºC, although obviously body temperature *per se* could not be controlled. Following the elimination of suspect data, assessment of the BP waveform was conducted using a computer algorithm that automatically identified the maximum (SBP) and minimum (DBP) points for each cardiac cycle. These points were visually checked and subsequently corrected where necessary. As the data were analysed on a beat-by-beat basis and periods where artefact occurred were discarded, the data were minimally affected by cuff changes and automatic calibration intervals.

The data were collected using a 12-channel *Grass* Model 7D pen-chart recorder (Grass Instrument Co., Quincy, MA, USA). The signals were amplified, filtered with a 50 Hz notch filter and displayed on paper chart. The ECG bandwidth was within 0.03 Hz-75 Hz and the high frequency filter for BP was set at 75 Hz. The rate of digitisation was 2000 Hz for both BP and ECG. Selected signals were also recorded on a Pentium IV PC via a 12 bit A/D converter using an acquisition program developed within the laboratory.

Assessment of the arterial baroreflex

The characterisation of BR activity is controversial with substantial disagreement between authorities both as to the validity of different methods of assessment (19, 32) and as to the nature of BR control (9, 21). Further, pharmacologic methods considered to be the "gold standard" could not be applied in the current study as they do not have the time resolution to identify changes within the SO period. Consequently, we have used two methods to assess participants' BR activity in order to obtain convergence of results. However, we recognise each has its limitations and these will be considered in the Discussion section.

The first method was the sequence technique (19, 24, 30, 31). This procedure identified series of consecutive beats that showed progressive increases in both SBP and pulse interval (PI) or decreases in both (+SBP/+PI, -SBP/-PI, respectively). The minimum criterion for a change in SBP was 1 mmHg and for changes in PI, 4 msec. The changes had to be maintained for at least three beats and could occur with a lag in PI of either zero, one or two beats. Having identified all sets of these beats, two measures of BR activity were assessed. First, the average regression of PI on SBP during these sequences, and second, the rate of sequences, where 'rate' was calculated as the number of sequences obtained within a phase divided by the total time spent in the phase. For both of these calculations, only sequences with linear r^2 values   0.85 were considered so that random variations in SBP and PI that appeared as sequences were not included in the computations.

The second approach to assess BR activity was via an autoregressive multivariate technique that used a bivariate algorithm to provide a closed-loop evaluation of the interactions between SBP and HR (3). PI and SBP values were converted into synchronized time series constructed at 3 Hz by use of a third order splining and filtering algorithm (Matlab). Time series of approximately 3 to 7 minutes were selected from each phase and were analysed by a bivariate autoregressive model of order 30, as described in Barbieri *et al.* (2001). For each signal, the spectral parameters were extracted using a division into a low frequency interval, ranging from 0.04 to 0.15 Hz, and a high frequency interval ranging from 0.15 to 0.5 Hz. Coherence between PI and SBP powers were computed using the cross-spectrum normalized by the two autospectral power densities. Indices were considered reliable for coherence values above 0.45. A coherence value of 0.45 was used rather than the 0.50

suggested by Taylor *et al*. (45) because we were evaluating relatively high frequencies (0.04 to 0.15 Hz) rather than very low frequencies, and were using parametric autoregressive estimation with a high autoregressive order of 30, rather than non-parametric estimation. The index of BR activity used was the average gain in the low frequency range (alpha gain).

Data Reduction

Because of the night-to-night variability in the duration of SO, the data were treated differently within each phase. Only phases 1 (pre-sleep wakefulness) and 5 (stable sleep) were of a constant length for all nights and all participants. These phases were of 30 mins duration and the data were averaged into 15 consecutive 2 min epochs. In phase 5, the intention was to determine the level to which BP and HR fell during sleep. As such, data during occasional arousals from sleep after stable sleep had been attained were discarded from the analysis. Phases 2, 3 and 4 did not have constant lengths across nights or across participants. Consequently, these data were averaged within proportions of the total time spent in the phase. Thus, the duration of each phase was divided into ten equal segments with one epoch representing 10% of the total time spent in the phase. BP and HR were also analysed using the total phase averages. BR activity, as assessed by the sequence technique, was analysed using phase averages, while in the autoregressive multivariate model, because it required longer time series, phases 3 and 4 were combined to give four phases.

To assess the effect of spontaneous arousals, BP and HR data were referenced to the onset of the arousal and averaged over arousals within participants, for phases 3 and 4, and for each arousal intensity. The temporal profile of BP and HR responses were represented graphically using the 20 pre and 20 post arousal beats. Statistically, the magnitude of the arousal response for each variable was defined in two ways. First, by comparing the mean of the 10 pre arousal beats with the mean of the 10 post arousal beats, and second, by comparing the mean of the 10 pre arousal beats with the maximum value in the 20 post arousal beats.

As would be anticipated, on some nights participants had a "smooth" transition to sleep such that phase 4 was not present (14 of 48 nights/10 participants). However, as there was night-to-night variability in absolute BP values, the data consequently showed abrupt artificial changes at phase 3

and 4, and phase 4 and 5 transitions due to the inclusion of a different subset of nights in phase 4 compared to all other phases. It was not considered appropriate to discard nights without phase 4 as an absence of this phase was a normal sleep event. Thus, the missing phase 4 values were estimated by interpolation, although degrees of freedom were appropriately adjusted in statistical analyses. Additionally, one participant had a shortened phase 5 and was excluded from the phase analyses but not the arousal analyses. In the autoregressive multivariate model of BR activity, where no data was available for a particular phase, that participant was excluded from the analysis rather than interpolating their missing data. Finally, just 14 participants had arousals at all intensities so only these participants were used for the intensity analyses. The number of participants and nights contributing to each analysis are indicated in the Table and Figure legends.

Statistical Analyses

All analyses were conducted across participants, with nights being averaged within participants. The effect of SO on SBP, DBP and HR was initially assessed by comparing the five phase averages in a one-way repeated measures ANOVA. Further, trend analysis was used to identify significant effects over epochs within phases. Using the sequence technique, BR activity was assessed by a 5 (phases) x 2 (sequence type, positive or negative) ANOVA with repeated measures on both variables. Data from the autoregressive multivariate model were analysed via a one-way repeated measures ANOVA with four levels (phases 3 and 4 being combined). Responses to arousal were assessed using a 2 (phases 3 and 4) by 2 (pre and post arousal or pre and max post arousal) ANOVA for SBP, DBP and HR. The impact of arousal intensity on these variables was assessed via a one-way repeated measures ANOVA with three intensity levels and the data averaged across phases 3 and 4. Finally, correlational analyses were conducted in order to explore the relationships between the occurrence of arousals, sleep and the changes in SBP, DBP and HR over SO.

RESULTS

The average time taken to reach stable sleep (phase 5) was 33.3 min and was highly variable between participants (range 6.3-100.5 min; SD, 22.0 min). The mean durations of phases 2, 3 and 4

were 12.2 $\pm$ 12.5 min, 8.1 $\pm$ 6.9 min, and 13.6 $\pm$ 15.2 min, respectively. The latency to the first

occurrence of stage 2 sleep, the conventional definition of sleep onset latency (SOL), was 20.3 min.

Comparison over phases

As shown in Figure 1, there were marked decreases in both SBP and DBP from pre-sleep

wakefulness to stable sleep, although the changes were not progressive over the SO phases. From

relaxed wakefulness to stable sleep, SBP fell by approximately 12 mmHg and DBP by 7 mmHg, with

the minimum values (SBP: 94.1, DBP: 46.4 mmHg) occurring approximately 15 mins into NREM

sleep. A one-way repeated measure ANOVA conducted over the five phase means revealed that these

decreases were significant (SBP: $F_{4,76}$=21.617, p<.001; DBP: $F_{4,76}$=10.549, p<.001). To clarify the

pattern of change in BP, pair-wise comparisons between phase averages with Bonferroni corrections

for multiple comparisons were conducted (Table 2). For SBP, these comparisons were all significant

except from phases 3 to 4 and 3 to 5 where SBP increased (though not significantly), and between

phases 2 and 4 where SBP was falling after LO. For DBP, the only significant pair-wise comparisons

were between the outermost phases that compared wakefulness with stable sleep, and between phase 1

and the initial onset of sleep (phase 3) (Table 2). There were no progressive changes in DBP across

phases reflecting a more gradual decline over SO than for SBP. However, for both SBP and DBP,

there were significantly marked declines over epochs within phase 2, that is after LO but before the

onset of stage 1 sleep (SBP: $F_{9,171}$=3.53, p<.001; DBP: $F_{9,171}$=2.05, p<.05). Further, in phase 5 once

stable sleep was attained, SBP but not DBP fell significantly (SBP: $F_{14,266}$=3.84, p<.001; DBP:

$F_{14,266}$=1.64, p>.05). Importantly, while BP appeared to decrease throughout the pre-sleep

wakefulness period, an analysis over epochs within phase 1 indicated that these reductions were not

significant (SBP: $F_{14,266}$=0.90, p>.05; DBP: $F_{14,266}$=0.50, p>.05). Despite some variability in the fall

over time, trend analysis indicated a significant linear component in the decrease in BP over the five

phases (SBP: $F_{1,19}$=58.235, p<.001; DBP: $F_{1,19}$=25.905, p<.001).

[Insert Figure 1 and Table 2]

HR declined by a total of 6.6 beats/min from pre-sleep wakefulness to stable sleep, showing an abrupt although transient increase at LO (Figure 1). With the transient removed (the last value in phase 1 and the first two values in phase 2), there was a significant fall in HR across epochs in phase 1, indicating a slow decline in the pre-sleep wakefulness period ($F_{13,247}=1.84$, p<.05). Beginning late in phase 4, HR reached its minimum value at the onset of stable stage 2 sleep (phase 5) and then fluctuated around 62 beats/min for the remainder of the phase. A repeated measures ANOVA conducted on the five phase averages indicated a significant decrease in HR over SO ($F_{4,76}=32.75$, p<.001). As indicated in Table 2 and correcting for multiple comparisons, the difference in HR from phase 1 to 2 and the slight reduction from phase 3 to 4 were not significant, however all other pair-wise comparisons between phase averages were significant. As with BP, trend analysis indicated a significant linear decrease in HR across SO ($F_{1,19}=70.92$, p<.001).

Assessment of BR function

Figure 2 describes BR activity over SO, as measured by both the sequence technique and autoregressive multivariate model. The rate of occurrence of sequences gradually increased from approximately 5 to 6 per min from phase 1 to phase 5 and was higher over successive SO phases, except in phase 3 where it underwent a temporary dip to pre-sleep wakefulness levels (Figure 2). The change in the rate of sequences was significant ($F_{4,76}=2.984$, p<.05) with a significant linear trend ($F_{1,19}=4.461$, p<.05). However, pair-wise differences between phase means were all not significant after applying Bonferroni corrections, thus reflecting the small change in BR activity with SO (Table 2). Further, the rate of negative sequences (-SBP/-PI) was greater than that of positive sequences (+SBP/+PI) ($F_{1,19}=11.05$, p<.01), although the interaction effect was not significant ($F_{4,76}=2.38$, p>.05). The slope of the BR showed an increase of approximately 4 msec/mmHg from phase 1 to 4 and then levelled off once stable sleep was attained, although these changes were not significant ($F_{4,76}=2.23$, p>.05). The slope of the BR did not differ between sequence type ($F_{1,19}=4.15$, p>.05) and the interaction between phase and type was not significant ($F_{4,76}=1.69$, p>.05) (see Figure 2).

[Insert Figure 2]

Using the autoregressive multivariate model to assess BR activity over SO, the alpha gain showed a rapid increase of approximately 3 msec/mmHg from phase 1 to 2 in association with turning the lights off (Figure 2). From phases 2 to 4, the alpha gain remained unchanged before decreasing about 1 msec/mmHg with the attainment of stable sleep. These changes across SO were significant ($F_{3,48}$=3.07, p<.05) showing significant linear ($F_{1,16}$=6.16, p<.05) and quadratic trends ($F_{1,16}$=5.27, p<.05) with the effect sizes being approximately the same for both components (linear: ^{2}=0.28; quadratic: ^{2}=0.25). The only significant differences in pair-wise comparisons (with Bonferroni corrections) were between phase 1 and 2 of SO when the lights were turned off (Table 2).

BP and HR responses to arousal during phases 3 and 4

There were a total of 435 spontaneous arousals identified, 204 in phase 3 and 231 in phase 4. The mean number of arousals per participant was 10 in phase 3 and 11 in phase 4, while the average rate of arousals per minute was 0.49 for phase 3 and 0.77 for phase 4.

Arousals in both phases 3 and 4 caused transient increases in BP and HR, beginning immediately after the onset of the arousal (Figure 3 and Table 3). Although the influence of an arousal appeared greater in phase 4 for SBP, the arousal response in phases 3 and 4 did not differ significantly for SBP, DBP or HR. This was indicated by an absence of interaction effects between phases and the means of the 10 pre and 10 post beats (SBP: $F_{1,19}$=3.347, p>.05; DBP: $F_{1,19}$=2.819, p>.05; HR: $F_{1,19}$=2.552, p>.05), and also the means of the 10 pre with the maximum in the 20 post beats (SBP: $F_{1,19}$=2.227, p>.05; DBP: $F_{1,19}$=1.663, p>.05; HR: $F_{1,19}$=1.577, p>.05). Of interest was the observation that while the pre arousal level for each variable approximated phase 3 and 4 average levels, the peak of the response approximated phase 1 (wakefulness) levels (Figure 3).

[Insert Figure 3 and Table 3]

When analysing the cardiovascular response to arousal as a function of arousal intensity, seven participants who did not have arousals at each level of intensity were discarded. 348 arousals remained of which 78 were level 1, 216 were level 2, and 54 were level 3 intensity. The mean number

of arousals for each participant at each intensity level was 6, 15, and 4 for arousal intensities 1, 2 and 3, respectively.

Table 4 shows that more intense arousals caused larger increases in SBP, DBP and HR (SBP: $F_{2,26}=22.21$, p<.001; DBP: $F_{2,26}=19.82$, p<.001; HR: $F_{2,26}=16.43$, p<.001). Pair-wise comparisons between arousal intensities with Bonferroni corrections for multiple comparisons indicated that the magnitude of level 1 arousals was significantly less than level 3 arousals (SBP: $F_{1,13}=30.877$, p<.001; DBP: $F_{1,13}=29.244$, p<.001; HR: $F_{1,13}=16.523$, p<.01), but not level 2 arousals (SBP: $F_{1,13}=1.823$, p>.01; DBP: $F_{1,13}=0.313$, p>.01; HR: $F_{1,13}=0.215$, p>.01), whereas the magnitude of level 3 arousals was significantly greater than level 2 arousals (SBP: $F_{1,13}=26.377$, p<.001; DBP: $F_{1,13}=22.420$, p<.001; HR: $F_{1,13}=17.999$, p<.01).

[Insert Table 4]

Relationship between arousals, sleep and the change in cardiovascular activity over SO

Correlational analyses were conducted to determine the relationship between the occurrence of arousals, SOL, and the total fall and rate of fall of BP and HR. Arousals were quantified as both the number and frequency per minute during phases 3 and 4. To capture the degree of fall in BP and HR, SOL was defined as the time from LO to the onset of stable sleep (beginning of phase 5). The falls in BP and HR were defined as both the total fall (phase 1 mean minus the last 10% epoch of phase 4) and the rate of fall per minute (total fall / SOL). The correlation coefficients are presented in Table 5. They indicate that a high number of arousals were significantly associated with a longer SOL and both a smaller fall and a slower rate of fall in BP and HR. Further, participants who went to sleep quicker had larger falls and a significantly faster rate of fall in BP and HR. This pattern was more apparent for BP than for HR. Rate of arousals (number/min) generally showed the same pattern although the effects did not reach significance. Finally, there was not a close relationship between the number and rate of arousals.

[Insert Table 5]

DISCUSSION

The most notable aspect of the data was the dependence of BP and HR on the phase of SO. Over the total SO period, BP was substantially reduced early in stable sleep compared with pre-sleep wakefulness, confirming several past reports suggesting a strong sleep effect occurring abruptly at SO (8, 14, 48, 50). Furthermore, the present data indicated that the fall in BP occurred during two discrete phases of SO. Initially, immediately after LO when participants were still awake, and secondly, as was hypothesised, following the attainment of stable sleep. As predicted, from the onset of stage 1 sleep until stable sleep, the fall in BP was retarded, particularly SBP, most likely as a consequence of the sleep-wake state instability that characterises this period. The effect of SO on HR was similar to BP in showing a general tendency to decrease over the SO period. However, HR demonstrated several although perhaps minor idiosyncratic features compared with BP. First, HR showed a transient increase at LO. Second, HR continued to fall during phase 3. Lastly, the fall in HR with the attainment of stable stage 2 sleep was more rapid than the fall in BP at this time. There was some suggestion that BR activity increased over the SO period. There was a significant increase in alpha gain and in the rate of both positive and negative sequences, however the slope of the sequences did not significantly change. These results were broadly consistent with earlier findings indicating greater BR sensitivity during NREM sleep compared to wakefulness (12, 30, 31, 42), and suggests that similar to BP and HR, changes in BR activity are initiated during the SO period. Importantly, there was no suggestion that BR activity decreased and thus the reduction in BP and HR do not appear to be a consequence of reduced sensitivity in the system.

While the observation that BP and HR fell before the occurrence of sleep was not anticipated, it was consistent with previous studies that had tentatively identified changes in anticipation of sleep (10, 14, 37). Further, the present study identified LO as the instigating event although it did not identify the causal mechanism(s). There are however a number of possibilities. First, although body position was controlled by having subjects lay supine, subtle changes in posture or muscle tone may have occurred in association with LO. Second, LO may be associated with a change in cognitive set from a focus on wakefulness to a focus on sleep, with attendant changes in physiology. It is unclear whether allowing subjects to read, listen to music or watch television or videos would have enhanced

such an effect, although these procedures were introduced to assist subjects to remain awake. However, it remains unclear whether such activities would have had a greater effect on cardiovascular activity than subjects' unaided efforts to remain awake. Third, it has been proposed that light directly affects HR such that a reduction in illumination would reduce cardiac activity (38, 39). Indeed, Scheer *et al.* (39) showed that administration of light to human participants increased HR, although the effect was circadian phase dependent, being more marked in the morning than the evening. Fourth, the fall in BP may have occurred as a consequence of peripheral vasodilation. It is well known that vasodilation occurs under circadian influence in anticipation of sleep (by several hours) and that sleep or sleep-related behaviour has an added influence (17). These changes are thought to primarily reflect thermoregulatory processes, although they might also be influenced by a shift in autonomic nervous system balance to vagal dominance in anticipation of sleep (7) or at SO (8, 48).

As noted in the Methods section, there is not wide agreement as to the validity of various methods of assessing BR function. With reference to the current measures, there is dispute regarding the validity of the sequence method (19, 32) and ambiguity with autoregressive methods in deciding if the data has sufficient coherence to be confidently interpreted (45). Despite these difficulties, we believe the present data provide information of BR function over the SO period. Thus, while the different measures do not show an identical pattern, the general thought of the data suggests that BR activity either remains constant, or increases during SO. This conclusion is consistent with the existent data on the effects of stable sleep on BR activity (12, 30, 31, 42) and suggests that whatever BP level the system is defending, it does so with undiminished vigour. From this perspective, the simultaneous falls in BP and HR are likely to be due to some form of resetting of the BR, a conclusion consistent with the research of Bristow *et al.* (5), who using a pharmacologic method, reported downward resetting during established sleep. However, the current data do not allow us to determine whether during SO, this involves a resetting of the set point on the BR curve, or a shift of the curve to a lower level. Nor does the data indicate whether receptor or central resetting is involved (9). Nevertheless, we speculate that whatever the mechanism, the time course of the simultaneous falls in BP and HR over the SO period suggest that resetting of the BR is a consequence of unloading the system (9, 21), while the fall in BP (unloading) is due to a direct intervention of sleep mechanisms.

As the cardiovascular response to arousal from sleep has been extensively studied (6, 13, 26), the observation that brief arousals were accompanied by substantial, transient elevations in BP and HR was not a novel finding. The critical observation in the present study was that repetitive arousal from sleep appeared to retard the progressive fall in BP during phases 3 and 4, and the fall in HR during phase 4. Indeed, the effect of arousal from sleep was such that after LO, cardiac activity fell more under conditions of sustained wakefulness than during sleep disrupted by transient awakenings. Retardation of the fall in BP and HR was not simply due to transient responses contributing to elevated averages. Rather, the mean phase values and the pre-arousal values were similar (see Figure 3) and did not fall below the level achieved at the end of phase 2. This pattern indicates first, that the transient responses were too brief to have a noticeable effect on the average BP and HR levels, and second, that the underlying fall in sleep values for BP and HR were retarded by arousals from sleep.

Correlational analyses also suggested a critical influence of arousal from sleep. Participants who aroused a large number of times had smaller reductions in BP, and to some extent HR. However, as these two variables were both correlated with SOL (long SOL latencies being associated with a greater number of arousals and smaller reductions in BP and HR), cause and effect was difficult to determine. Nevertheless, overall the data suggested that following an initial fall after LO, BP and HR did not fall further until stable sleep was achieved. This conclusion is consistent with the view that the "non dipper" BP profile identified in OSA is in part due to frequent arousal from sleep (27, 44).

There is the possibility that the sleep transition process was altered by the measurement conditions, however the addition of the Portapres device to standard sleep recording techniques would not be considered a major intrusion on sleep (51). Combined with this, the mean SOL to stage 2 sleep was approximately 20 min, well within the normal range for a sleep laboratory night, although some participants did take considerable time to reach stable stage 2 sleep.

Previous work had suggested that BP and HR fall in association with SO. However, the temporal discrimination of these studies had not been sufficient to document either the close association or the complexity of the changes between SO and the fall in BP and HR. The present paper identified changes in both variables, which in terms of their sleep-related changes were completed soon after the establishment of stable sleep. The data also indicated that the changes were

dependent on the phase of SO and were initiated at LO, before the first EEG indications of sleep. The parallel changes in BP and HR in the absence of a reduction in BR activity suggested a downward resetting of the set point of the BR, most likely as a result of a sleep related unloading of the system. Finally, the data indicated that the fall in HR and particularly BP were suspended during those phases of SO characterised by repetitive arousal from sleep. This finding emphasises the importance of sleep stability for the development of the reduced cardiac activity thought to be characteristic of NREM sleep.

GRANTS

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REFERENCES

1.   **ASDA.** EEG arousals: scoring rules and examples: a preliminary report from the Sleep Disorders Atlas Task Force of the American Sleep Disorders Association. *Sleep* 15: 173-184, 1992.

2.   **Athanassiadis D, Draper GJ, Honour AJ, and Cranston WI.** Variability of automatic blood pressure measurements over 24 hour periods. *Clin Sci* 36: 147-156, 1969.

3.   **Barbieri R, Parati G, and Saul JP.** Closed- versus open-loop assessment of heart rate baroreflex. *IEEE Eng Med Biol Mag* 20: 33-42, 2001.

4.   **Bevan AT, Honour AJ, and Stott FH.** Direct arterial pressure recording in unrestricted man. *Clin Sci* 36: 329-344, 1969.

5.   **Bristow JD, Honour AJ, Pickering TG, and Sleight P.** Cardiovascular and respiratory changes during sleep in normal and hypertensive subjects. *Cardiovasc Res* 3: 476-485, 1969.

6.   **Burgess HJ, Kleiman J, and Trinder J.** Cardiac activity during sleep onset. *Psychophysiology* 36: 298-306, 1999.

7.   **Burgess HJ, Trinder J, Kim Y, and Luke D.** Sleep and circadian influences on cardiac autonomic nervous system activity. *Am J Physiol* 273: H1761-1768, 1997.

8.   **Carrington M, Walsh M, Stambas T, Kleiman J, and Trinder J.** The influence of sleep onset on the diurnal variation in cardiac activity and cardiac control. *J Sleep Res* 12: 213-221, 2003.

9.   **Chapleau MW, Hajduczok G, and Abboud FM.** Resetting of the arterial baroreflex: Peripheral and central mechanisms. In: *Reflex control of the circulation*, edited by Zucker IH and Gilmore JP. Boca Raton: CRC Press, 1991, p. 165-198.

10.   **Coccagna G, Mantovani M, Brignani F, Manzini A, and Lugaresi E.** Arterial pressure changes during spontaneous sleep in man. *Electroencephalogr Clin Neurophysiol* 31: 277-281, 1971.

11.   **Colrain IM, Trinder J, Fraser G, and Wilson GV.** Ventilation during sleep onset. *J Appl Physiol* 63: 2067-2074, 1987.

12.   **Conway J, Boon N, Jones JV, and Sleight P.** Involvement of the baroreceptor reflexes in the changes in blood pressure with sleep and mental arousal. *Hypertension* 5: 746-748, 1983.

13.   **Davies RJ, Belt PJ, Roberts SJ, Ali NJ, and Stradling JR.** Arterial blood pressure responses to graded transient arousal from sleep in normal humans. *J Appl Physiol* 74: 1123-1130, 1993.

14.   **Degaute JP, Van de Borne P, Linkowski P, and Van Cauter E.** Quantitative analysis of the 24-hour blood pressure and heart rate patterns in young men. *Hypertension* 18: 199-210, 1991.

15.   **Kay A, Trinder J, Bowes G, and Kim Y.** Changes in airway resistance during sleep onset. *J Appl Physiol* 76: 1600-1607, 1994.

16.   **Kerkhof GA, Van Dongen HP, and Bobbert AC.** Absence of endogenous circadian rhythmicity in blood pressure? *Am J Hypertens* 11: 373-377, 1998.

17.   **Krauchi K and Wirz-Justice A.** Circadian clues to sleep onset mechanisms. *Neuropsychopharmacology* 25: S92-96, 2001.

18.   **Krauchi K and Wirz-Justice A.** Circadian rhythm of heat production, heart rate, and skin and core temperature under unmasking conditions in men. *Am J Physiol* 267: R819-829, 1994.

19.   **Lipman RD, Salisbury JK, and Taylor JA.** Spontaneous indices are inconsistent with arterial baroreflex gain. *Hypertension* 42: 481-487, 2003.

20.   **Littler WA, Honour AJ, Carter RD, and Sleight P.** Sleep and blood pressure. *Br Med J* 3: 346-348, 1975.

21.   **Lohmeier TE, Irwin ED, Rossing MA, Serdar DJ, and Kieval RS.** Prolonged activation of the baroreflex produces sustained hypotension. *Hypertension* 43: 306-311, 2004.

22.   **Mancia G, Ferrari A, Gregorini L, Parati G, Pomidossi G, Bertinieri G, Grassi G, Di Rienzo M, Pedotti A, and Zanchetti A.** Blood pressure and heart rate variabilities in normotensive and hypertensive human beings. *Circ Res* 53: 96-104, 1983.

23.   **Mann S, Millar-Craig MW, Melville DI, Balasubramanian V, and Raftery EB.** Physical activity and the circadian rhythm of blood pressure. *Clin Sci* 57: 291s-294s, 1979.

24.   **Mateika JH, Kavey NB, and Mitru G.** Spontaneous baroreflex analysis in non-apneic snoring individuals during NREM sleep. *Sleep* 22: 461-468, 1999.

25.   **Millar-Craig MW, Bishop CN, and Raftery EB.** Circadian variation of blood-pressure. *Lancet* 1: 795-797, 1978.

26.   **Morgan BJ, Crabtree DC, Puleo DS, Badr MS, Toiber F, and Skatrud JB.** Neurocirculatory consequences of abrupt change in sleep state in humans. *J Appl Physiol* 80: 1627-1636, 1996.

27.   **Noda A, Okada T, Hayashi H, Yasuma F, and Yokota M.** 24-hour ambulatory blood pressure variability in obstructive sleep apnea syndrome. *Chest* 103: 1343-1347, 1993.

28.   **O'Brien E, Sheridan J, and O'Malley K.** Dippers and non-dippers. *Lancet* 2: 397, 1988.

29.   **Palatini P, Penzo M, Racioppa A, Zugno E, Guzzardi G, Anaclerio M, and Pessina AC.** Clinical relevance of nighttime blood pressure and of daytime blood pressure variability. *Arch Intern Med* 152: 1855-1860, 1992.

30.   **Parati G, Di Rienzo M, Bertinieri G, Pomidossi G, Casadei R, Groppelli A, Pedotti A, Zanchetti A, and Mancia G.** Evaluation of the baroreceptor-heart rate reflex by 24-hour intra-arterial blood pressure monitoring in humans. *Hypertension* 12: 214-222, 1988.

31.   **Parati G, Di Rienzo M, Bonsignore MR, Insalaco G, Marrone O, Castiglioni P, Bonsignore G, and Mancia G.** Autonomic cardiac regulation in obstructive sleep apnea syndrome: evidence from spontaneous baroreflex analysis during sleep. *J Hypertens* 15: 1621-1626, 1997.

32.   **Parati G, Di Rienzo M, Castiglioni P, Bouhaddi M, Cerutti C, Cividjian A, Elghozi JL, Fortrat JO, Girard A, Janssen BJ, Julien C, Karemaker JM, Iellamo F, Laude D, Lukoshkova E, Pagani M, Persson PB, Quintin L, Regnard J, Ruediger JH, Saul PJ, Vettorello M, Wesseling KH, and Mancia G.** Assessing the sensitivity of spontaneous baroreflex control of the heart: deeper insight into complex physiology. *Hypertension* 43: e32-34, 2004.

33.   **Penaz J.** Photoelectric measurement of blood pressure, volume and flow in the finger. *Digest 10th Int Conf Med Biol Engng* (104 ed.), Dresden, 1973.

34.   **Pickering GW, Sleight P, and Smyth HS.** The relation of arterial pressure to sleep and arousal in man. *J Physiol (Lond)* 191: 76P-78P, 1967.

35.   **Pivik RT and Busby K.** Heart rate associated with sleep onset in preadolescents. *J Sleep Res* 5: 33-36, 1996.

36.   **Rechtschaffen A and Kales A.** A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects. Washington, D.C.: National Institute of Health, 1968.

37.   **Richardson DW, Honour AJ, Fenton GW, Stott FH, and Pickering GW.** Variation in arterial pressure throughout the day and night. *Clin Sci* 26: 445-460, 1964.

38.   **Scheer FA, Ter Horst GJ, Van Der Vliet J, and Buijs RM.** Physiological and anatomic evidence for regulation of the heart by suprachiasmatic nucleus in rats. *Am J Physiol Heart Circ Physiol* 280: H1391-1399, 2001.

39.   **Scheer FA, Van Doornen LJ, and Buijs RM.** Light and diurnal cycle affect human heart rate: Possible role for the circadian pacemaker. *J Biol Rhythms* 14: 202-212, 1999.

40.   **Sforza E, Jouny C, and Ibanez V.** Cardiac activation during arousal in humans: Further evidence for hierarchy in the arousal response. *Clin Neurophysiol* 111: 1611-1619, 2000.

41.   **Sforza E, Nicolas A, Lavigne G, Gosselin A, Petit D, and Montplaisir J.** EEG and cardiac activation during periodic leg movements in sleep: support for a hierarchy of arousal responses. *Neurology* 52: 786-791, 1999.

42.   **Smyth HS, Sleight P, and Pickering GW.** Reflex regulation of arterial pressure during sleep in man. A quantitative method of assessing baroreflex sensitivity. *Circ Res* 24: 109-121, 1969.

43.   **Snyder F, Hobson JA, Morrison DF, and Goldfrank F.** Changes in respiration, heart rate, and systolic blood pressure in human sleep. *J Appl Physiol* 19: 417-422, 1964.

44.   **Somers VK, Dyken ME, Clary MP, and Abboud FM.** Sympathetic neural mechanisms in obstructive sleep apnea. *J Clin Invest* 96: 1897-1904, 1995.

45.   **Taylor JA, Carr DL, Myers CW, and Eckberg DL.** Mechanisms underlying very-low-frequency RR-interval oscillations in humans. *Circulation* 98: 547-555, 1998.

46.   **Trinder J, Allen N, Kleiman J, Kralevski V, Kleverlaan D, Anson K, and Kim Y.** On the nature of cardiovascular activation at an arousal from sleep. *Sleep* 26: 543-551, 2003.

47.   **Trinder J, Kay A, Kleiman J, and Dunai J.** Gender differences in airway resistance during sleep. *J Appl Physiol* 83: 1986-1997, 1997.

48.   **Trinder J, Kleiman J, Carrington M, Smith S, Breen S, Tan N, and Kim Y.** Autonomic activity during human sleep as a function of time and sleep stage. *J Sleep Res* 10: 253-264, 2001.

49.   **Van de Borne P, Biston P, Paiva M, Nguyen H, Linkowski P, and Degaute JP.** Cardiorespiratory transfer during sleep: a study in healthy young men. *Am J Physiol* 269: H952-958, 1995.

50.   **Van de Borne P, Nguyen H, Biston P, Linkowski P, and Degaute JP.** Effects of wake and sleep stages on the 24-h autonomic control of blood pressure and heart rate in recumbent men. *Am J Physiol* 266: H548-554, 1994.

51.   **Van de Borne P, Nguyen H, Linkowski P, and Degaute JP.** Sleep quality and continuous, non-invasive beat-to-beat blood pressure recording. *J Hypertens* 11: 1423-1427, 1993.

52.   **Van den Meiracker AH, Man in 't Veld AJ, Van Eck HJ, Wenting GJ, and Schalekamp MA.** Determinants of short-term blood pressure variability. Effects of bed rest and sensory deprivation in essential hypertension. *Am J Hypertens* 1: 22-26, 1988.

53.   **Van Dongen HP, Maislin G, and Kerkhof GA.** Repeated assessment of the endogenous 24-hour profile of blood pressure under constant routine. *Chronobiol Int* 18: 85-98, 2001.

54.   **Verdecchia P, Schillaci G, Gatteschi C, Zampi I, Battistelli M, Bartoccini C, and Porcellati C.** Blunted nocturnal fall in blood pressure in hypertensive women with future cardiovascular morbid events. *Circulation* 88: 986-992, 1993.

55.   **Worsnop C, Kay A, Pierce R, Kim Y, and Trinder J.** Activity of respiratory pump and upper airway muscles during sleep onset. *J Appl Physiol* 85: 908-920, 1998.
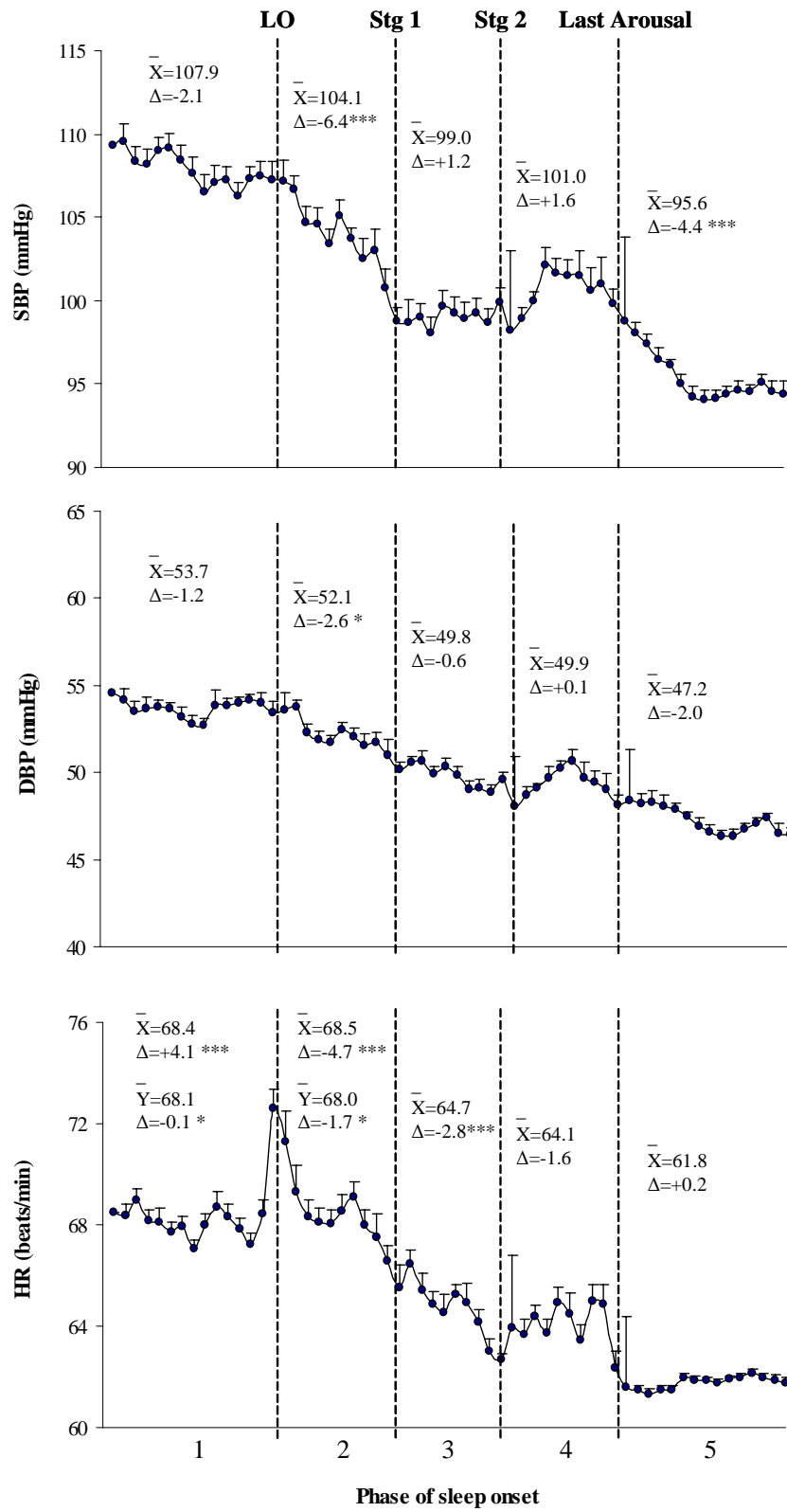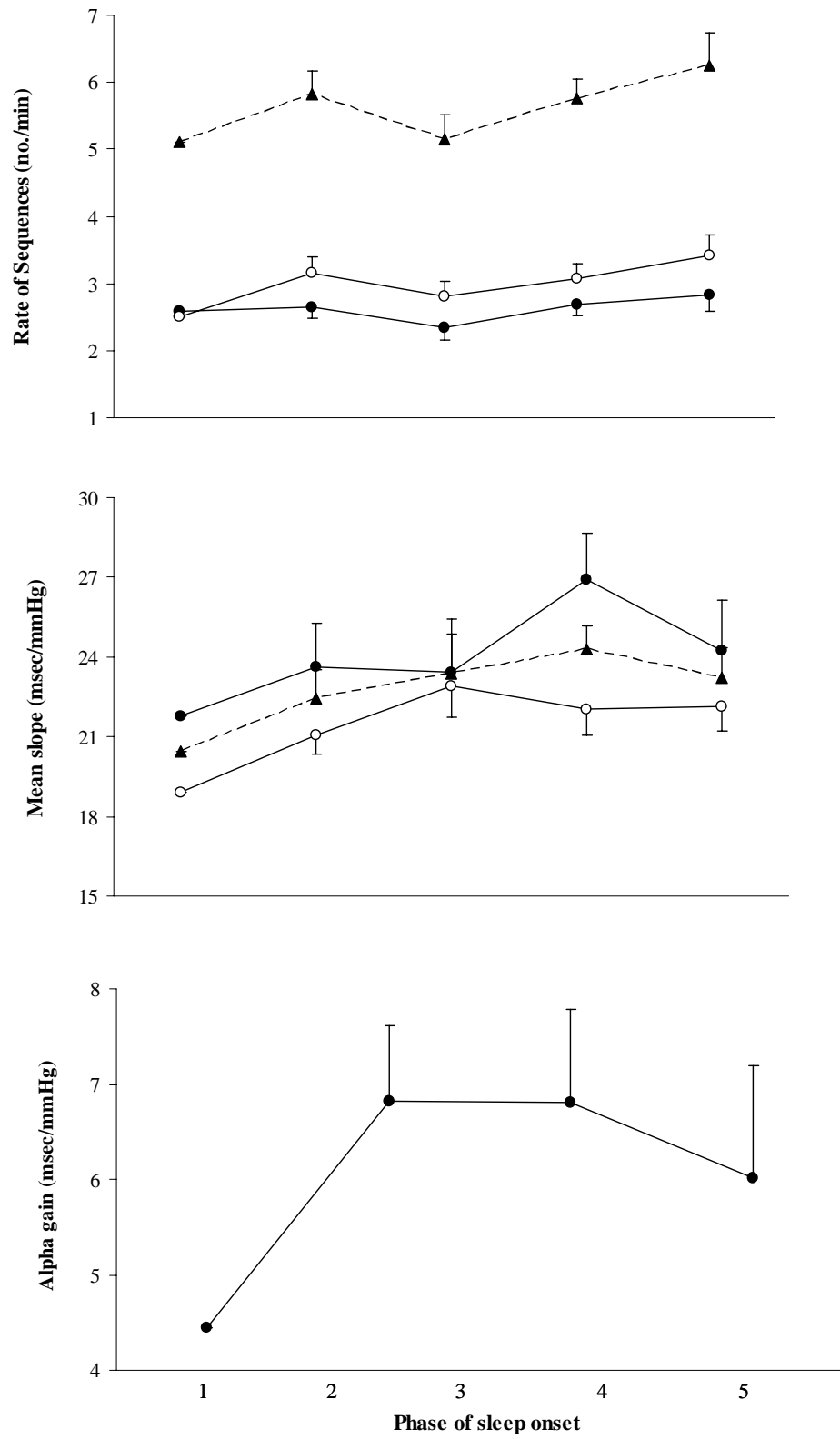
Figure Legends

Figure 1.   Mean SBP, DBP and HR over sleep onset as a function of time (2 min epochs) in phases 1 and 5, and 10% epochs in phases 2, 3 and 4. SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; mmHg, millimeters of mercury; LO, lights out; $\overline{X}$, overall phase average; $\overline{Y}$, phase average excluding the last value before LO and the first two values following LO (for HR data only); Δ, change from the beginning to the end of the phase; *, p < 0.05; ***, p < 0.001. N=20 (46 nights). Standard error bars indicate within subject variability (variance in the change within subjects over time).

Figure 2.   Mean baroreflex activity over phases of sleep onset as represented by the rate of sequences and slope within sequences using the sequence technique, and the alpha gain of the autoregressive multivariate technique. No./min, number per minute; msec/mmHg, milliseconds per millimeters of mercury;   , positive sequences (+SBP/+PI);   , negative sequences (-SBP/-PI); -▲-, positive and negative sequences combined (for sequence technique data only). Phases 3 and 4 are combined for the alpha gain data. N=20 (46 nights) except for the alpha gain data where N=17 (41 nights). Standard error bars indicate within subject variability (variance in the change within subjects over time).

Figure 3.   Mean SBP, DBP and HR response to arousal from phases 3 and 4 of sleep onset. SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; mmHg, millimeters of mercury; , phase 3;   , phase 4. Labelled values on the right indicate phase 1, 3 and 4 averages. N=20 (43 nights). Standard error bars indicate within subject variability (variance in the change within subjects over time).

LO **Stg 1** **Stg 2** **Last Arousal**

$\bar{X}=107.9$
$\Delta=-2.1$

$\bar{X}=104.1$
$\Delta=-6.4$***

$\bar{X}=99.0$
$\Delta=+1.2$

$\bar{X}=101.0$
$\Delta=+1.6$

$\bar{X}=95.6$
$\Delta=-4.4$ ***

$\bar{X}=53.7$
$\Delta=-1.2$

$\bar{X}=52.1$
$\Delta=-2.6$ *

$\bar{X}=49.8$
$\Delta=-0.6$

$\bar{X}=49.9$
$\Delta=+0.1$

$\bar{X}=47.2$
$\Delta=-2.0$

$\bar{X}=68.4$
$\Delta=+4.1$ ***

$\bar{Y}=68.1$
$\Delta=-0.1$ *

$\bar{X}=68.5$
$\Delta=-4.7$ ***

$\bar{Y}=68.0$
$\Delta=-1.7$ *

$\bar{X}=64.7$
$\Delta=-2.8$***

$\bar{X}=64.1$
$\Delta=-1.6$

$\bar{X}=61.8$
$\Delta=+0.2$

SBP (mmHg)

DBP (mmHg)

HR (beats/min)

Phase of sleep onset

Arousal Onset
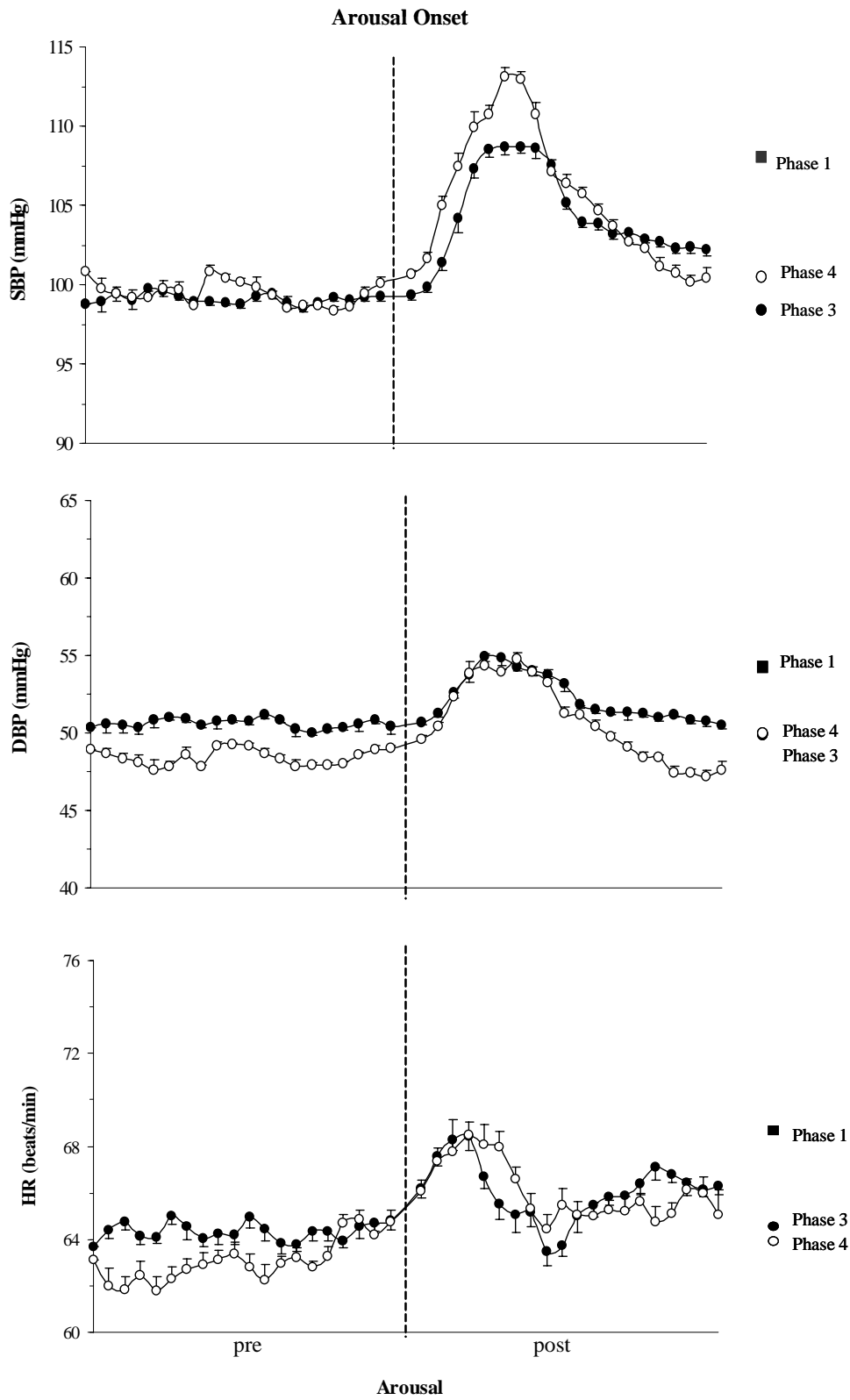
Table 1.   Three-level arousal classification system

Arousal Intensity Level	Arousal Scoring Criteria
1	a) EEG slow waves, submental EMG increase, EOG activity, but no increase in EEG frequency (alpha or faster). b) EEG change (alpha or faster) lasting 1.5-3 sec, with/without submental EMG increase or EOG activity.
2	EEG change (alpha or faster) with duration of 3 sec, with/without sub-mental EMG increase or EOG activity, but without gross body movements.
3	EEG change (alpha or faster) lasting 3 sec, with submental EMG increase or EOG activity and gross body movements.

EEG, electroencephalogram; EMG, electromyogram; EOG, electrooculogram.

Table 2. *F* - ratios for all pair-wise comparisons between phase means for SBP, DBP, HR and baroreflex data

Comparison	SBP	DBP	HR	Rate of sequences	Slope	Alpha gain
Phase 1 v 2	10.59 *	2.04	0.02	4.14	3.55	8.76 *
Phase 1 v 3	33.50 *	12.16 *	38.57 *	0.03	7.28	
Phase 1 v 4	20.38 *	7.65	23.11 *	6.91	9.30 *	
Phase 1 v 3&4						8.24
Phase 1 v 5	57.37 *	25.52 *	83.14 *	6.29	2.95	4.77
Phase 2 v 3	13.97 *	6.57	39.00 *	3.24	0.42	
Phase 2 v 4	4.98	3.95	20.07 *	0.02	1.24	
Phase 2 v 3&4						0.00
Phase 2 v 5	33.69 *	25.15 *	65.47 *	0.52	0.16	0.95
Phase 3 v 4	3.82	0.01	0.91	4.99	1.05	
Phase 3 v 5	5.71	6.94	31.60 *	6.13	0.01	
Phase 3&4 v 5						0.46
Phase 4 v 5	10.05 *	6.14	14.26 *	1.08	0.87	

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate. N=20 (46 nights), df (1,19) for all variables except alpha gain where N=17 (41 nights), df (1,16). *, $p < 0.01$ (the *per comparison* error rate after Bonferroni adjustment). Although changes in the slope of the BR over SO was not significant, pair-wise comparisons between phases are displayed for contrast.

Table 3. Mean pre and post arousal levels and arousal magnitude for phase 3, phase 4 and phases 3 and 4 combined for SBP, DBP and HR

	SBP (mmHg)			DBP (mmHg)			HR (beats/min)		
	Phs 3	Phs 4	Phs 3 & 4 combined	Phs 3	Phs 4	Phs 3 & 4 combined	Phs 3	Phs 4	Phs 3 & 4 combined
$\overline{X}$ 10 pre	99.11	99.12	99.12	50.21	48.39	49.30	64.85	63.58	64.22
	(2.07)	(2.51)		(1.40)	(1.69)		(2.29)	(2.35)	
Max [20] post	110.67	113.72	112.19	56.13	55.88	56.01	71.59	72.30	71.94
	(3.00)	(3.13)		(1.72)	(2.07)		(2.17)	(2.67)	
Arousal magnitude	11.56	14.60	13.07	5.92	7.49	6.71	6.74	8.72	7.72

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; mmHg, millimetres of mercury. Values in brackets are the standard error of the mean (SEM). N=20 (43 nights).

Table 4.　Mean pre and post arousal levels and arousal magnitude for intensity levels 1, 2 and 3 for SBP, DBP and HR

| | SBP (mmHg) | | | DBP (mmHg) | | | HR (beats/min) | | |
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
$\overline{X}$ 10 pre	101.89	100.38	96.73	51.78	51.45	45.46	62.30	63.51	64.05
Max [20] post	108.82	110.11	118.79	54.51	54.94	57.43	67.88	69.62	85.13
Arousal magnitude	6.93	9.74	22.06	2.73	3.49	11.96	5.58	6.10	21.08
	(1.83)	(2.09)	(2.87)	(0.86)	(1.32)	(1.33)	(0.76)	(0.77)	(3.54)

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; mmHg, millimetres of mercury. Values in brackets represent the SEM. N=14 (31 nights).

Table 5.　Correlation coefficients between the number and rate of arousals, SOL and changes in SBP, DBP and HR

	Arousal number	Rate of arousal	SOL	Rate of fall of SBP, DBP & HR over SO
Rate of arousal	.260			
SOL	.501 *	.264		
Rate of fall of SBP, DBP & HR over SO	SBP: -.517 * DBP: -.398 HR: -.453 *	SBP: -.136 DBP: .116 HR: -.073	SBP: -.670 ** DBP: -.621 ** HR: -.663 **	
% change over SO	SBP: -.630 ** DBP: -.724 ** HR: -.515 *	SBP: -.430 DBP: -.141 HR: -.153	SBP: -.415 DBP: -.562 ** HR: .000	SBP: .698 ** DBP: .645 ** HR: .343

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; SOL, sleep onset latency; SO, sleep onset.　N=20 (46 nights).　*, $p < 0.05$; **, $p < 0.01$.