Changes in cardiovascular function during the sleep onset period in young adults

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BLOOD PRESSURE (BP) and heart rate (HR) fluctuate over 24 h, being lower during nighttime 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, whereas 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 rest studies that permit sleep while 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, whereas the remaining sleep period is associated with a gradual rise toward 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 nighttime 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 behavior, 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. Furthermore, it remains unclear whether cardiovascular activity is influenced by a critical physiological “switch” event indexed by α-θ EEG changes, as observed in the respiratory system (11, 15, 55). Such an influence would predict that periodic reappearance of EEG α-activity, indicative of arousal from sleep and accompanied by substantial transient elevations that return BP and HR to approximately presleep wakefulness levels (6, 13, 26), would impede the sleep-related decreases in these variables. However, whereas 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 “nondipping” 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 nondipping 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 presleep 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 to provide a description of the impact of EEG changes on cardiovascular function. We hypothesized 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 10 men and 11 women with a mean age of 21.1 ± 2.8 yr (range 18–26 yr). Their average body mass index was 22.9 ± 2.1 kg/m² (range 19.3–26.9 kg/m²). All participants were healthy nonsmokers who had not undertaken any shift work or transmeridian travel in the 3 mo before 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. Furthermore, their sleep records were scrutinized for evidence of any sleep-disordered breathing. Last, 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. Furthermore, 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 (3 participants), two (9 participants), or three (9 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 the paper chart recordings. Phase 1 consisted of a 30-min period of relaxed wakefulness before 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 s of continuous θ-activity. Phase 3 was the time between the first 10 s of continuous θ-activity and the first sleep spindle or K complex. Phase 3 incorporated alternating periods of α- and θ-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 θ-activity after 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 after the last arousal in phase 4. This phase was characterized by continuous stage 2 or slow-wave sleep and by the absence of transient arousals.

Cardiac activity was analyzed 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 characterized by sleep-wake state instability, arousals from sleep were identified to assess their effect on cardiovascular activity. Arousals can vary from relatively brief electromyographic (EMG) increases (eliciting noncortical activation) to increases in cortical EEG desynchronized 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 was 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 one to three, with lower grade arousals representing subtle/microarousals, level 2 arousals meeting the American Sleep Disorders Association 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 s of θ-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 analyzed as a function of phases 3 or 4 and the three arousal intensities.

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 before 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 presleep 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 ~50 lx and 22–24°C, 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 (10 participants) or until their first sleep cycle was completed (11 participants).

Table 1. Three-level arousal classification system

<table>
<thead>
<tr>
<th>Arousal Intensity Level</th>
<th>Arousal Scoring Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>a) EEG slow waves, submental EMG increase, EOG activity, but no increase in EEG frequency (α or faster).</td>
</tr>
<tr>
<td>2</td>
<td>EEG change (α or faster) lasting 1.5–3 s, with/without submental EMG increase or EOG activity, but without gross body movements.</td>
</tr>
<tr>
<td>3</td>
<td>EEG change (α or faster) lasting ≥3 s, with submental EMG increase or EOG activity and gross body movements.</td>
</tr>
</tbody>
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Assessment of sleep-wake state. Participants’ sleep-wake state was assessed according to standardized sleep recordings and procedures (36). They consisted of a central (C3-A2) and occipital (O1-A2) EEG, a submental EMG, and an EOG (left and right outer canthi offset from horizontal).

Assessment of HR. Participants’ HR was assessed with an ECG measured through three Meditrace Ag-AgCl spot electrodes (Graphic Controls, Buffalo, NY). 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 interbeat 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 BP. BP was assessed by the arterial volume-clamp method (33), providing a continuous noninvasive 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 ischemic discomfort associated with continuous cuff inflation by regularly alternating measurements between two fingers. Theuffed fingers were maintained at heart level throughout the recording procedure, except for a small number of participants who read during the presleep wakefulness period. This was not considered critical as the equipment incorporated an automated height adjustment.

The BP data were inspected for body movement artifact 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 night of recording lost. Additionally, because peripheral arterial tone of the finger is affected by temperature, the laboratory temperature was set at 22–24°C, although obviously body temperature per se could not be controlled. After 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 analyzed on a beat-by-beat basis and periods where artifact 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, QuinCy, MA). The signals were amplified, filtered with a 50-Hz notch filter, and displayed on paper chart. The ECG bandwidth was within 0.03–75 Hz, and the high-frequency filter for BP was set at 75 Hz. The rate of digitization was 2,000 Hz for both BP and ECG. Selected signals were also recorded on a Pentium IV personal computer via a 12-bit analog-to-digital converter using an acquisition program developed within the laboratory.

Assessment of the arterial BR. The characterization of BR activity is controversial, with substantial disagreement between authorities as to both the validity of different methods of assessment (19, 32) and the nature of BR control (9, 21). Furthermore, pharmacological 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 used two methods to assess participants’ BR activity to obtain convergence of results. However, we recognize each has its limitations, and these will be considered in the Discussion.

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 was 4 ms. 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 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 $\geq 0.85$ 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 3–7 min were selected from each phase and were analyzed by a bivariate autoregressive model of order 30, as described in Barbieri et al. (3). 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. Indexes were considered reliable for coherence values $>0.45$. 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 we were using parametric autoregressive estimation with a high autoregressive order of 30, rather than nonparametric 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 (presleep wakefulness) and 5 (stable sleep) were of a constant length for all nights and all participants. These phases were of 30-min 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 10 equal segments, with one epoch representing 10% of the total time spent in the phase. BP and HR were also analyzed using the total phase averages. BR activity, as assessed by the sequence technique, was analyzed using phase averages, whereas 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 10 pre- and 20 postarousal beats. Statistically, the magnitude of the arousal response for each variable was defined in two ways: first, by comparing the mean of the 10 prearousal beats with the mean of the 10 postarousal beats, and second, by comparing the mean of the 10 prearousal beats with the maximum value in the 20 postarousal 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 with 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 were available for a particular phase, that participant was excluded from the analysis rather than interpolating the 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 legends of Tables 2–5 and Figs. 1–3.

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. Furthermore, trend analysis was used to identify significant effects over epochs within phases. With the sequence technique, BR activity was assessed by a 5 (phases) × 2 (sequence type, positive or negative) ANOVA with repeated measures on both variables. Data from the autoregressive multivariate model were analyzed 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) × 2 (pre- and postarousal or pre- and maximum postarousal) 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 were averaged across phases 3 and 4. Finally, correlational analyses were conducted 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 ± 12.5 min, 8.1 ± 6.9 min, and 13.6 ± 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 Fig. 1, there were marked decreases in both SBP and DBP from presleep wakefulness to stable sleep, although the changes were not progressive over the SO phases. From relaxed wakefulness to stable sleep, SBP fell by ~12 mmHg and DBP by 7 mmHg, with the minimum values (SBP: 94.1, DBP: 46.4 mmHg) occurring ~15 min into NREM sleep. A one-way repeated-measures ANOVA conducted over the five-phase means revealed that these decreases were significant (SBP: $F_{4.76} = 21.617, P < 0.001$; DBP: $F_{4.76} = 10.549, P < 0.001$). To clarify the pattern of change in BP, pairwise 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 (although 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: $F_{9.171} = 3.53, P < 0.001$; DBP: $F_{9.171} = 2.05, P < 0.05$). Furthermore, in phase 5 once stable sleep was attained, SBP but not DBP fell significantly (SBP: $F_{14.266} = 3.84, P <...
Table 2. F ratios for all pairwise comparisons between phase means for SBP, DBP, HR, and baroreflex data

<table>
<thead>
<tr>
<th>Comparison</th>
<th>SBP</th>
<th>DBP</th>
<th>HR</th>
<th>Rate of Sequences</th>
<th>Slope</th>
<th>α-Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1 vs. 2</td>
<td>10.59*</td>
<td>2.04</td>
<td>0.02</td>
<td>4.14</td>
<td>3.55</td>
<td>8.76*</td>
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<tr>
<td>Phase 1 vs. 3</td>
<td>33.50*</td>
<td>12.16*</td>
<td>38.57*</td>
<td>0.03</td>
<td>7.28</td>
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<tr>
<td>Phase 1 vs. 4</td>
<td>20.38*</td>
<td>7.65</td>
<td>23.11*</td>
<td>6.91</td>
<td>9.30*</td>
<td></td>
</tr>
<tr>
<td>Phase 1 vs. 3 and 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.24</td>
</tr>
<tr>
<td>Phase 1 vs. 5</td>
<td>57.37*</td>
<td>25.52*</td>
<td>83.14*</td>
<td>6.29</td>
<td>2.95</td>
<td>4.77</td>
</tr>
<tr>
<td>Phase 2 vs. 3</td>
<td>13.97*</td>
<td>6.57</td>
<td>39.00*</td>
<td>3.24</td>
<td>0.42</td>
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<td>Phase 2 vs. 4</td>
<td>4.98</td>
<td>3.95</td>
<td>20.07*</td>
<td>0.02</td>
<td>1.24</td>
<td></td>
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<tr>
<td>Phase 2 vs. 3 and 4</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>Phase 2 vs. 5</td>
<td>33.69*</td>
<td>25.15*</td>
<td>65.47*</td>
<td>0.52</td>
<td>0.16</td>
<td>0.95</td>
</tr>
<tr>
<td>Phase 3 vs. 4</td>
<td>3.82</td>
<td>0.01</td>
<td>0.91</td>
<td>4.99</td>
<td>1.05</td>
<td></td>
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<tr>
<td>Phase 3 vs. 5</td>
<td>5.71</td>
<td>6.94</td>
<td>31.60*</td>
<td>6.13</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Phase 3 and 4 vs. 5</td>
<td>10.05*</td>
<td>6.14</td>
<td>14.26*</td>
<td>1.08</td>
<td>0.87</td>
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</tr>
<tr>
<td>Phase 4 vs. 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.46</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate. n = 20 (46 nights), df (1,19) for all variables except α-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 baroreflex over sleep onset (SO) was not significant, pairwise comparisons between phases are displayed for contrast.

SBP, DBP, HR, and the rate of sequences and slope within sequences using the sequence technique, trend analysis indicated a significant linear component in the decrease in BP over the five phases (SBP: F_{14,266} = 9.0, P > 0.05; DBP: F_{14,266} = 0.5, P > 0.05). Despite some variability in the fall over time, trend analysis indicated a significant linear decrease in HR across SO (Table 2). Furthermore, the rate of negative sequences (+SBP/−DBP) was greater than that of positive sequences (+SBP/+DBP) (F_{1,19} = 11.05, P < 0.01), although the interaction effect was not significant (F_{4,76} = 2.38, P > 0.05). The slope of the BR showed an increase of ~4 ms/mmHg from phases 1 to 4 and then leveled off once stable sleep was attained, although these changes were not significant (F_{4,76} = 2.23, P > 0.05). The slope of the BR did not differ between sequence type (F_{1,19} = 4.15, P > 0.05), and the interaction between phase and type was not significant (F_{4,76} = 1.69, P > 0.05; see Fig. 2).

Using the autoregressive multivariate model to assess BR activity over SO, the α-gain showed a rapid increase of ~3

![Image of Figure 2](https://jap.physiology.org/)

**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 α-gain of the autoregressive multivariate technique. PI, pulse interval. 
- Positive sequences (+SBP/−DBP); ○, negative sequences (−SBP/+DBP); ▲, positive and negative sequences combined (for sequence technique data only). Phases 3 and 4 are combined for the α-gain data. n = 20 (46 nights) except for the α-gain data, where n = 17 (41 nights). SE bars indicate within-subject variability (variance in the change within subjects over time).
Arousal Onset

ms/mmHg from phase 1 to 2 in association with turning the lights off (Fig. 2). From phases 2 to 4, the $\alpha$-gain remained unchanged before decreasing $-1$ ms/mmHg with the attainment of stable sleep. These changes across SO were significant ($F_{3,48} = 3.07, P < 0.05$) showing significant linear ($F_{1,16} = 6.16, P < 0.05$) and quadratic trends ($F_{1,16} = 5.27, P < 0.05$) with the effect sizes being approximately the same for both components (linear: $\eta^2 = 0.28$; quadratic: $\eta^2 = 0.25$). The only significant differences in pairwise comparisons (with Bonferroni corrections) were between phases 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, whereas 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 (Fig. 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 postarousal beats (SBP: $F_{1,19} = 2.819, P > 0.05$; DBP: $F_{1,19} = 2.227, P > 0.05$; HR: $F_{1,19} = 2.552, P > 0.05$), and also the means of the 10 pre- with the maximum in the 20 postarousal beats (SBP: $F_{1,19} = 2.227, P > 0.05$; DBP: $F_{1,19} = 1.663, P > 0.05$; HR: $F_{1,19} = 1.577, P > 0.05$). Of interest was the observation that, whereas the prearousal level for each variable approximated phase 3 and 4 average levels, the peak of the response approximated phase 1 (wakefulness) levels (Fig. 3).

When the cardiovascular response to arousal as a function of arousal intensity was analyzed, data from seven participants who did not have arousals at each level of intensity were discarded. Three hundred forty-eight 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: $F_{2,26} = 22.21, P < 0.001$; DBP: $F_{2,26} = 19.82, P < 0.001$; HR: $F_{2,26} = 16.43, P < 0.001$). Pairwise 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: $F_{1,13} = 30.877, P < 0.001$; DBP: $F_{1,13} = 29.244, P < 0.001$; HR: $F_{1,13} = 16.523, P < 0.01$), but not level 2 arousals (SBP: $F_{1,13} = 1.823, P > 0.05$; DBP: $F_{1,13} = 0.313, P > 0.05$; HR: $F_{1,13} = 0.215, P > 0.05$), whereas the magnitude of level 3 arousals was significantly greater than level 2 arousals (SBP: $F_{1,13} = 26.377, P < 0.001$; DBP: $F_{1,13} = 22.420, P < 0.001$; HR: $F_{1,13} = 17.999, P < 0.01$).

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

<table>
<thead>
<tr>
<th></th>
<th>SBP, mmHg</th>
<th>DBP, mmHg</th>
<th>HR, beats/min</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Phase 3</td>
<td>Phase 4</td>
<td>Combined</td>
</tr>
<tr>
<td>Mean 10 pre</td>
<td>99.11 (2.07)</td>
<td>99.12 (2.51)</td>
<td>99.12</td>
</tr>
<tr>
<td>Maximum (20) post</td>
<td>110.67 (3.00)</td>
<td>113.72 (3.13)</td>
<td>112.19</td>
</tr>
<tr>
<td>Arousal magnitude</td>
<td>11.56</td>
<td>14.60</td>
<td>13.07</td>
</tr>
</tbody>
</table>

Values in parentheses are the SE. $n = 20$ (43 nights).
Table 4. Mean pre- and postarousal levels and arousal magnitude for intensity levels 1, 2, and 3 for SBP, DBP, and HR

<table>
<thead>
<tr>
<th>SBP, mmHg</th>
<th>DBP, mmHg</th>
<th>HR, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>Level 2</td>
<td>Level 3</td>
</tr>
<tr>
<td>Mean 10 pre</td>
<td>101.89</td>
<td>100.38</td>
</tr>
<tr>
<td>Maximum (20) post</td>
<td>108.82</td>
<td>110.11</td>
</tr>
<tr>
<td>Arousal magnitude</td>
<td>6.93 (1.83)</td>
<td>9.74 (2.09)</td>
</tr>
</tbody>
</table>

Values in parentheses represent the SE. n = 14 (31 nights).

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. Furthermore, 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.

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 presleep 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 second, as was hypothesized, after 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 characterizes 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. Last, 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 α-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 with 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 does not appear to be a consequence of reduced sensitivity in the system.

Although 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). Furthermore, 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

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

<table>
<thead>
<tr>
<th>Arousal Number</th>
<th>Rate of Arousal</th>
<th>SOL</th>
<th>Rate of Fall of SBP, DBP, and HR Over SO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of arousal</td>
<td>0.260</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOL</td>
<td>0.501*</td>
<td>0.264</td>
<td></td>
</tr>
<tr>
<td>Rate of fall of SBP, DBP, and HR over SO</td>
<td>SBP: −0.517*</td>
<td>SBP: −0.136</td>
<td>SBP: −0.670†</td>
</tr>
<tr>
<td>DBP: −0.398</td>
<td>DBP: 0.116</td>
<td>DBP: −0.621†</td>
<td></td>
</tr>
<tr>
<td>HR: −0.453*</td>
<td>HR: −0.073</td>
<td>HR: −0.663†</td>
<td></td>
</tr>
<tr>
<td>%Change over SO</td>
<td>SBP: −0.630†</td>
<td>SBP: −0.430</td>
<td>SBP: −0.415</td>
</tr>
<tr>
<td>DBP: −0.724†</td>
<td>DBP: −0.141</td>
<td>DBP: −0.562</td>
<td></td>
</tr>
<tr>
<td>HR: −0.515*</td>
<td>HR: −0.153</td>
<td>HR: 0.000</td>
<td></td>
</tr>
</tbody>
</table>

SOL, sleep onset latency. n = 20 (46 nights). *P < 0.05. †P < 0.01.
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 behavior 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 METHODS, 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 have 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, whereas 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 existing 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 vigor. 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 do 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 suggests that resetting of the BR is a consequence of unloading the system (9, 21), whereas 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 prearousal values were similar (see Fig. 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 awoke a large number of times had smaller reductions in BP and, to some extent, HR. However, because these two variables were both correlated with SOL (long SOL 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 more until stable sleep was achieved. This conclusion is consistent with the view that the “nondipper” 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 ~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 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 falls in HR and particularly BP were suspended during these phases of SO characterized by repetitive arousal from sleep. This finding emphasizes 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

CARdiovascular Activity During Sleep Onset in Young Adults


