On the Nature of Cardiovascular Activation at an Arousal from Sleep

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Study Objectives: The intent of the study was to explore the nature and function of the cardiovascular activation response that occurs at an arousal from sleep.

Design: Four experiments were conducted. The first compared the pattern of physiologic response to orienting and startle stimuli and arousal from sleep. The second and third measured the amplitude of the cardiovascular arousal response as a function of the trait of fearfulness and the threat value of the arousing stimulus, respectively. The final experiment assessed the effect of arousal duration.

Setting: The experiments were conducted in the sleep laboratory of the Department of Psychology, University of Melbourne.

Participants: A total of 42 (24 women and 18 men) healthy individuals between the ages of 18 and 24 participated in the experiments.

Interventions: The experiments manipulated the stimuli to which participants were exposed (orienting and startle stimuli and arousal from sleep), the threat value of stimuli used to arouse participants from sleep, and individual differences in fearfulness.

Measurements and Results: The major dependent variables were heart rate, blood pressure, and a measure of peripheral vasoconstriction (digital pulse volume). In addition, in the first study, the galvanic skin response and orbicularis oculi electromyographic activity were measured. Experiment 1 showed that the pattern of physiologic response at an arousal from sleep differed, with a substantially larger cardiovascular component, from responses to orienting and startle stimuli. Experiments 2a and 2b indicated that the magnitude of the cardiovascular response at an arousal was unrelated to either individual differences in fearfulness or differences in the threat value of arousing stimuli. The final experiment showed that the cardiovascular response at an arousal was not a return to waking levels of activity but, rather, was a transient activation response.

Conclusions: The study supported the view that the cardiovascular activation response at an arousal from sleep is a transient, reflex-like response that is different from the response that occurs during normal wakefulness.

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INTRODUCTION

AROUSAL FROM SLEEP IN HUMANS IS ASSOCIATED WITH SUBSTANTIAL CARDIOVASCULAR ACTIVATION. The most notable effects are increases in heart rate (HR), blood pressure (BP), and peripheral vasoconstriction.1,2 Because stroke volume appears to decrease slightly and cardiac output changes little,2 it is likely that the increase in BP primarily reflects peripheral vasoconstriction. There has been considerable interest in the nature of this cardiovascular activation response. This is because a number of sleep disorders are associated with frequent arousal from sleep and because it has been suggested that the associated cardiovascular activation, and the resultant swings in BP, may have pathophysiologic consequences for the cardiovascular system.3

However, while the activation response has been well described (eg, in Morgan et al’s work3), little is known about its function, eliciting stimuli, and factors that may affect the amplitude of the activation response. One hypothesis is that it is an evolved, species-specific reflex that has the function of preparing the arousing sleeper to interact with the environment.4,5 Consistent with this, it has been suggested that the activation response is specifically associated with arousal from sleep6 and that it is elicited by a component of the act of arousing, rather than by the stimulus that caused the arousal.3 Further, it has been suggested that the state during an arousal is different from normal wakefulness,7 such that the waking response may be independent of homeostatic mechanisms that might be activated at this time.3,7,8

The above hypothesis, what might be termed the evolutionary hypothesis, suggests a highly stereotyped response that is specific to arousal from sleep and is modifiable only by a restricted class of stimuli. The present paper reports results from 4 studies designed to explore 3 different aspects of the hypothesis with a view to better understanding its nature and function and, potentially, its role in pathophysiologic processes.

The first study assessed whether the response is a version of the orienting reflex (OR) or the startle reflex (SR),4,5 rather than a reflex specifically associated with arousal from sleep (here referred to as the waking reflex [WR]). The research strategy adopted was to compare patterns of physiologic responding following orienting stimuli (OS), startle stimuli (SS), and spontaneous arousals from sleep. The second and third studies assessed whether the cardiovascular response to an arousal from sleep could be modified by environmental threat, given that this might constitute an ecologically valid stimulus. The studies assessed whether the activation response was larger when subjects were aroused by threatening stimuli and when the subjects were predisposed to interpret the environment as threatening. The final study assessed whether the timecourse of the activation response was dependent on how quickly the subject returned to sleep.

In order to clarify terminology, it should be noted that the OS and SS refer to standard stimuli employed in the psychophysiology literature to elicit the OR or SR, respectively. The term WR is suggested as a term to refer to the characteristic cardiovascular activation response that occurs at an arousal from sleep.

EXPERIMENT ONE

METHODS

Subjects

Twelve subjects (6 women; 6 men) with a mean age of 21.42 years (SD=2.43) and an average body mass index (BMI) of 22.8 kg/m² (SD=2.11) were recruited by advertisement from the student body of the University of Melbourne. Potential subjects were screened with a questionnaire for health problems, sleep disorders, family history of cardiovascular or respiratory disorders, smoking, current medications, exces-
sive caffeine (>350 mg per day) or alcohol consumption (>5 standard drinks per week), excessive exercise (>10 hours per week), and time-zone travel during the previous 3 months. The study was approved by the University of Melbourne’s Human Ethics Committee, and all subjects gave written informed consent prior to their participation.

Design

A number of physiologic measures were recorded in each subject in response to 3 types of stimuli or events: OS, SS, and spontaneous arousals from sleep. Data were collected immediately prior to and during subject’s normal sleep period. The series of OS and SS were presented with the subjects awake and supine in bed, beginning 2 hours and 1 hour before subject’s normal sleep-onset time. Spontaneous arousals from sleep were then collected during the normal sleep period. This sequence was maintained for all subjects because of concerns that if the SS had been presented first, the stimuli may have sensitized subjects to the OS and because it was logistically simpler to run the sleep condition last. The OS and SS conditions were not repeated following the collection of sleep data, as the sleep sessions generally concluded around 3:00 AM and subjects could not be kept awake for testing.

The physiologic variables collected were selected to document cardiovascular arousal and to reflect potentially idiosyncratic features of the response pattern to each stimulus or event. The measures were: HR, BP, digital pulse volume (PV) as a measure of vasoconstriction-dilation; galvanic skin response (GSR); and activity of the orbicularis oculi muscle (eye-blink response) via electromyography (EMG) (Ooemg).

Procedures

General Laboratory Procedures

Subjects were required to refrain from alcohol and caffeine consumption for 24 hours prior to their participation in the study. They arrived at the laboratory 3.5 hours before their normal sleep-onset time. After subjects prepared for bed, the recording equipment was attached, and the subjects were put to bed. Subjects were kept in the supine position in all conditions. After running the OS and SS conditions, the light was turned out at the subject’s normal sleep-onset time, and the subjects were requested to go to sleep.

The OS and SS were generated by computer and delivered to subjects via E-A-RTONE™ 3A insert earphones.

Orienting Stimuli

The OS was a 70-decibel (dB) tone with instantaneous rise time and a duration of 500 milliseconds. A series of 20 tones was presented with a randomly ordered interstimulus interval of 30, 45, or 60 seconds. The 20 tones were divided into 4 types in order to slow the rate of habituation of the reflex: 1000-hertz (Hz) and 200-Hz tones presented to either the right or left ear. While all 4 tone types were included in the first 4 stimuli, the probability of a response was determined, where a response occurred within 2 seconds of the stimulus. In addition, the probability of an eye blink occurring with in 250 milliseconds of stimulus onset (OS and SS conditions) was determined, as were ± 250 milliseconds and ± 2 seconds of an arousal (sleep conditions). For the probability analysis, an arbitrary amplitude was used to define the occurrence of a blink within the specified time window.

The eye-blink SR was measured by recording the activity of the orbicularis oculi muscle below the right eye. Two gold-plated GRASS electrodes were placed horizontally over the muscle approximately 1 centimeter (cm) apart. The EMG activity was integrated by moving time average with a sampling rate of 200 Hz and a window of 100 milliseconds. The resulting power within R-R intervals was adjusted for R-R duration and analyzed beat by beat. Three measures were derived: the average integrated value, the peak integrated value, and the probability of a response.

Startle Stimuli

The SS consisted of white noise at 100 dB with instantaneous rise time and a duration of 50 milliseconds. Twenty tones were administered, with the interstimulus interval varying randomly between 30, 45, and 60 seconds.

Spontaneous Arousals from Sleep

As spontaneous arousal events occur relatively frequently during sleep onset and the early sleep period and occur infrequently once stable sleep is achieved, subjects were aroused by the experimenter after approximately 5 minutes of stable stage 2 sleep. Subjects were then kept awake for several minutes before being allowed to fall back to sleep.

This procedure has been used extensively in previous studies. A minimum of 4 hours of recording was collected for this condition.

Spontaneous arousals from sleep were identified from the sleep recordings by an experienced scorer (JT). The criteria were based on American Sleep Disorders Association (ASDA) recommendations, requiring 3 seconds of continuous electroencephalogram (EEG) alpha activity. In addition, a minimum of 20 seconds of continuous sleep, as defined by EEG theta activity, with or without sleep spindles and K-complexes, had to have occurred before an arousal could be scored. The point of onset of each arousal was defined by the first occurrence of either the onset of 3 seconds of alpha activity or the onset of other activity (an awake eye movement, submental EMG activity, a K complex) that resolved into at least 3 seconds of alpha activity. Finally, if a body movement or other artifact occurred during the arousal, the event was discarded.

Measures

Sleep-wake state was identified from EEG (C3-A2, O1-A2), submental EMG and electrooculogram (EOG) recordings, according to standard procedures. The EEG and EOG signals were filtered below 0.3 and above 30 Hz, and the EMG below 10 and above 100 Hz.

Assessment of HR was achieved via an electrocardiogram (ECG), recorded through Meditrace Ag/AgCl spot electrodes placed on the subject’s lower left and right rib cage and right clavicle. The HR was calculated from R-R intervals as determined automatically by computer algorithm. The BP was measured by a continuous finger BP recorder (Portapres, Model 2), with alternating cuffs placed on the second and third digits of the left hand, and an automated height-adjustment mechanism that corrected for changes in the position of the subject’s fingers with respect to their heart. Systolic (SBP) and diastolic (DBP) values were automatically determined. A finger photoplethysmograph, placed on the thumb of the right hand, was used to measure PV, which was defined in terms of the maximum value for each pulse on an arbitrary scale. Maximum values were identified automatically by computer algorithm. Each cardiac variable was analyzed on a beat-by-beat basis.

The eye-blink SR was measured by recording the activity of the orbicularis oculi muscle below the right eye. Two gold-plated GRASS electrodes were placed horizontally over the muscle approximately 1 centimeter (cm) apart. The EMG activity was integrated by moving time average with a sampling rate of 200 Hz and a window of 100 milliseconds. The resulting power within R-R intervals was adjusted for R-R duration and analyzed beat by beat. Three measures were derived: the average integrated value, the peak integrated value, and the probability of a response. In addition, the probability of an eye blink occurring within 250 milliseconds of stimulus onset (OS and SS conditions) was determined, as were ± 250 milliseconds and ± 2 seconds of an arousal (sleep conditions). For the probability analysis, an arbitrary amplitude was used to define the occurrence of a blink within the specified time window.

The GSR was measured using GRASS Ag/AgCl finger electrodes placed on the volar surface of the medial phalanges of the second and third digits of the right hand. The recording was scored from the paper chart. The amplitude of the response was defined as the difference in conductance (µSiemens) between the peak value within 10 seconds after the stimulus (or arousal) and the value at the time of the stimulus. In addition, the probability of a response was determined, where a response was defined as a 1% increase in conductance, within 10 seconds of the stimulus. All measures were amplified and filtered using a Grass model 7D Pen Chart Recorder. The EEG, EOG, submental EMG, ECG, BP, and GSR were recorded on paper at 10 millimeters per second, while the O1-A2 EEG ECG, and BP were also collected on computer. The Ooemg and finger plethysmograph were only recorded on computer. The EEG, BP, and finger plethysmograph were sampled at 100 Hz and the ECG and Ooemg at 1000 Hz. All computer-aided analyses were visually edited and manually corrected where necessary.
Data Reduction and Analysis

Initial inspection of the OR data suggested the HR bradycardia response to the stimulus had habituated by the sixth trial, and, thus, the values for this condition were limited to the first 5 trials. In the startle condition, when determining response amplitude, trials were only included if the trial contained a scorable eye-blink response. These procedures were introduced as these responses are definitive aspects of each reflex and it may thus be argued that the reflex had not been elicited in the absence of these responses.

The number of artifact-free arousal responses identified in the sleep recordings ranged from 19 to 68 over subjects. These were divided into 2 categories—those occurring early in sleep onset, before the occurrence of spindles and K complexes, and those occurring late in sleep onset, after the appearance of these events. This distinction was made because earlier unpublished work had suggested that the magnitude of the activation response increased as sleep developed.

As indicated above, most measures were analyzed in a number of ways. However, as the results were essentially identical over different representations of the data, not all methods of analysis have been fully reported in the results section.

For variables that were represented as absolute, rather than probability or difference values, and analyzed on a beat-by-beat basis (HR, SBP, DBP, PV, and OOemg), 20 values were calculated before and after the stimulus (tone presentation or arousal). The first poststimulus beat was allocated to the R-R interval occurring at the onset of arousal or tone presentation. Values were averaged within subjects for each position with respect to the stimulus and then averaged over subjects. While 20 prestimuli and poststimuli beats were used for graphic representation, only 10 prestimuli and poststimuli values were used for statistical analyses, as this period was considered to better capture the reflex activation period.

In the statistical analyses, the prestimulus values were initially compared over the 3 conditions. Two analyses were then performed on all of the data sets to determine the effect of the stimuli. Thus, the differences between the mean of the 10 prestimulus values and both the mean and peak value for the 10 poststimulus values were compared over the conditions. Each analysis used a 1-way repeated measures ANOVA. Significant effects were then explored with posthoc comparisons between conditions.

As indicated above, probability data were analyzed in a number of ways. However, as the results were essentially identical over different representations of the data, not all methods of analysis have been fully reported in the results section.

For probability data, the probability of a response (GSR or eye-blink) for each subject was transformed into a z-score and then compared to the results of Experiment 3. The probability of an eye-blink response differed markedly between conditions, with a spontaneouss arousal response (see Table 2). Further, in data not presented in Table 2, the probability of an eye-blink response differed markedly between conditions. The probability of a response within 250 milliseconds of a stimulus was .86 to the SS, while it was negligible to the other 2 stimuli (.05, .02, and .01 for the OS and a spontaneous arousal early and late in sleep onset, respectively). When the window in which eye-blink responses could occur was extended to the spontaneous arousal condition to ± 2 seconds, the probability increased to .11 and .21 for the early and late sleep-onset conditions. However, the morphology of these responses suggested they were primarily spontaneous eye blinks.

Finally, a GSR was frequently observed in response to both OS (58%) and SS (66%) but less frequently in response to a spontaneous arousal.

RESULTS AND DISCUSSION

The results of the prestimulus analyses are presented in Table 1. As would be anticipated, cardiovascular activity was lower during the prestimulus period in the spontaneous-arousal condition when the subjects were asleep, although the difference was not significant for SBP. Further, the activity of obicularis oculi did not differ between the conditions. Statistical procedures, such as ANCOVA, that take such differences into consideration were not used because it was felt that prestimulus differences were an important aspect of the data and potentially provided an explanation for the pattern of the results (see Discussion).

Subjects demonstrated significantly larger cardiovascular responses to a spontaneous arousal from sleep than to either the OS or SS (see Table 2). This difference was significant for HR, PV, and SBP, both early and late in sleep onset and for mean and peak comparisons. The only exception was the peak comparison for PV early in sleep onset, and this approached significance. The data for HR and SBP are illustrated in Figures 1 and 2.

In contrast, the OOemg response was only observed in response to the SS, the integrated value being significantly higher than for a spontaneous arousal (see Table 2). Further, in data not presented in Table 2, the probability of an eye-blink response differed markedly between conditions. The probability of a response within 250 milliseconds of a stimulus was .86 to the SS, while it was negligible to the other 2 stimuli (.05, .02, and .01 for the OS and a spontaneous arousal early and late in sleep onset, respectively). When the window in which eye-blink responses could occur was extended to the spontaneous arousal condition to ± 2 seconds, the probability increased to .11 and .21 for the early and late sleep-onset conditions. However, the morphology of these responses suggested they were primarily spontaneous eye blinks.

Table 1—Mean (SD) prestimulus values for the 3 stimulus conditions, early, and late in sleep onset, in Experiment 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Prestimulus Mean</th>
<th>F ratios</th>
<th>df=2,22 (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WR (SD) OR (SD) SR (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, bpm</td>
<td>Early 60.3 (6.9) 63.8 (8.6) 64.3* (7.9)</td>
<td>3.85 (&lt;.05)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Late 58.9 (7.3) 63.8* (8.6) 64.3** (7.9)</td>
<td>7.41 (&lt;.01)</td>
<td></td>
</tr>
<tr>
<td>PV, au</td>
<td>Early 14.1 (5.3) 9.6** (5.3) 10.1* (5.9)</td>
<td>7.95 (&lt;.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Late 14.6 (6.2) 9.6** (5.3) 10.1* (5.9)</td>
<td>6.25 (&lt;.01)</td>
<td></td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>Early 92.4 (16.8) 100.5 (18.1) 101.3 (14.0)</td>
<td>1.97 (NS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Late 82.4 (20.3) 100.5 (18.1) 101.3 (14.0)</td>
<td>2.74 (NS)</td>
<td></td>
</tr>
<tr>
<td>OOemg, au</td>
<td>Early 1331 (877) 1081 (583) 1142 (836)</td>
<td>0.70 (NS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Late 1227 (858) 1081 (583) 1142 (836)</td>
<td>0.25 (NS)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1—Heart-rate response to spontaneous arousals from sleep early and late in sleep onset (top panel), and to startle stimuli (SS) and orienting stimuli (OS) (bottom panel). bpm, beats per minute; PV, pulse volume; au, arbitrary units; SBP, systolic blood pressure; mmHg, millimeters of mercury; OOemg, obicularis oculi electromyogram. Early and Late indicate early and late in sleep onset. Probability in parentheses refers to the main effect. Astarisks indicate probability associated with posthoc comparisons between WR, and OR and SR (*, P<.05; **, P<.01).
In summary, subjects showed vigorous cardiovascular changes in response to an arousal from sleep. The magnitude of the effect was large for both early and late in sleep onset (P<.001). However, while the magnitude of the change in GSR showed larger increases in conductance for both SS and OS than spontaneous arousal, the differences did not reach significance (Table 2).

One limitation of the study was that the order of conditions was not balanced over subjects. Although there were specific reasons for this, the design does allow an interpretation in terms of circadian influences. However, this was considered unlikely, as exploratory analyses did not show any change in the magnitude of the WR, as a function of time within the sleep period. Further, the circadian phase difference between the presentation of the OS and SS, and the initial arousals from sleep, was minimal.

One perspective of the evolutionary view regarding the functional significance of the activation response at an arousal from sleep is that it prepares an organism for fight or flight following an arousal. This suggests that the magnitude of the activation response would be a function of the degree of perceived threat in the environment. The alternative view within the context of the evolutionary model is that the response is elicited by the act of waking, such that it anticipates potential environmental circumstances. From the latter perspective, the initial reflex response would be independent of the subsequently perceived threat. In this study, these alternative hypotheses were assessed by manipulating the level of threat at the time of an arousal from sleep. In Experiment 2A, this was achieved by comparing the cardiovascular responses of individuals with high, versus low, levels of fearfulness at an arousal from sleep. In Experiment 2B, responses were measured following arousals elicited by stimuli with different threat values.

**Table 2—Mean (SD) prestimulus to poststimulus difference for the 3 stimulus conditions, early and late in sleep onset, in Experiment 1.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean Difference</th>
<th>Peak Difference</th>
<th>Fratios (df=2,22)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WR</td>
<td>OR</td>
<td>SR</td>
</tr>
<tr>
<td>HR, bpm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>1.47 (2.19)</td>
<td>-0.70* (1.51)</td>
<td>0.00* (1.01)</td>
</tr>
<tr>
<td>Late</td>
<td>2.18 (3.03)</td>
<td>-0.70** (1.51)</td>
<td>0.00* (1.01)</td>
</tr>
<tr>
<td>PV, au</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>-3.4 (2.1)</td>
<td>-1.4* (2.2)</td>
<td>-0.8** (0.6)</td>
</tr>
<tr>
<td>Late</td>
<td>-5.3 (3.2)</td>
<td>-1.4*** (2.2)</td>
<td>-0.8*** (0.6)</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>12.1 (6.6)</td>
<td>-0.7*** (7.1)</td>
<td>2.3*** (5.4)</td>
</tr>
<tr>
<td>Late</td>
<td>11.2 (11.7)</td>
<td>-0.7* (7.1)</td>
<td>2.3* (5.4)</td>
</tr>
<tr>
<td>Ooemg, au</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>-6 (43)</td>
<td>195* (228)</td>
<td>195* (228)</td>
</tr>
<tr>
<td>Late</td>
<td>11 (65)</td>
<td>195* (228)</td>
<td>195* (228)</td>
</tr>
<tr>
<td>GSR, µSiemens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>2.80 (2.8)</td>
<td>21.34 (34.3)</td>
<td>26.18 (41.0)</td>
</tr>
<tr>
<td>Late</td>
<td>3.79 (7.8)</td>
<td>21.24 (34.3)</td>
<td>26.18 (41.0)</td>
</tr>
</tbody>
</table>

WR, waking reflex; OR, orienting reflex; SR, startle reflex; HR, heart rate; bpm, beats per minute; PV, pulse volume; au, arbitrary units; SBP, systolic blood pressure; mmHg, millimeters of mercury; Ooemg, obicularis occuli electromyogram. Early and Late indicate early and late in sleep onset. Probability in parentheses refers to the main effect. Asterisks indicate probability associated with posthoc comparisons between WR, and OR and SR (*, P<.05; **, P<.01). GSR, galvanic skin response. Remaining abbreviations are as for Table 1.
EXPERIMENT 2A

METHODS

Subjects and Design

The study consisted of 2 groups, low-fear and high-fear individuals. The subjects were first-year psychology students at the University of Melbourne. Two hundred and ten students underwent a preliminary screening study consisting of a number of psychological tests, including the Fear Survey Schedule (FSS). Subjects were selected for subsequent participation based on their responses on the FSS. The low-fear group was selected from among individuals below the lower 15th percentile, and the high-fear group from those above the 85th percentile. In addition, subjects were screened as in Experiment 1.

Nine subjects who had been selected for the high-fear group chose not to participate in the study, offering some validation of the scale. The final sample consisted of 10 low-fear subjects (6 women; 4 men) with a mean FSS item score of 1.56 (SD=.12), mean age of 18.5 years (SD=1.27), and mean BMI of 22.5 kg/m² (SD=3.05) and 8 high-fear subjects (7 women & 1 man) with a mean FSS item score of 3.26 (SD=3.1), mean age of 19.1 years (SD=1.13) and BMI of 21.7 kg/m² (SD=4.9).

Administration of the FSS on the laboratory night confirmed the group assignments, with mean FSS scores of 1.53 and 3.03 for the low- and high-fear groups, respectively. The University of Melbourne's Human Ethics Committee approved the study, and participants gave written informed consent and were reimbursed for the laboratory component of their participation.

Procedures

The study compared the cardiovascular responses of the 2 groups to spontaneous arousals from sleep. In general, the laboratory procedures were the same as for Experiment 1. The data collection, sleep scoring, and arousal identification procedures were also the same as for the spontaneous arousal condition in the first experiment. The exception was that GSR and Ooemg recordings were not collected, leaving HR, BP, and PV as the dependent variables.

Data Reduction and Analysis

The data analyzed were the 20 heartbeats preceding and following each spontaneous arousal. Trials on which another arousal, a body movement, or other artifact occurred in the period beginning 30 seconds before the arousal or stimulus until the end of the 20 heartbeats after the arousal were discarded. The number of valid spontaneous arousals varied from 18 to 55, with an average of 36. Arousals from early and late in sleep onset were combined.

The HR, BP, and PV values were averaged at each heart-beat position over trials within subjects and then over subjects within groups. The 20 prearousal and postarousal values were displayed graphically, while 10 prearousal and postarousal values were analyzed statistically. As in Experiment 1, 2 analyses were conducted. In one, the mean of the 10 prearousal values were compared to the mean of the 10 postarousal values, while in the other, the mean prearousal value was compared to the peak postarousal value. The ANOVA model was a 2 (groups) by 2 (pre-post) factorial with repeated measures on the second factor.

RESULTS

Mean values and a summary of the statistical analyses are presented in Table 3. The data clearly indicate that the high-fear individuals did not have larger changes in cardiovascular activity at a spontaneous arousal from sleep. The data for HR are illustrated in the top panel of Figure 3. It should be noted that the statistical analysis indicated that the apparent group difference was not sta-
tistically significant (see Table 3).

**EXPERIMENT 2B**

**METHODS**

**Subjects and Design**

Eight subjects (4 women; 4 men), with mean age of 20.45 years (SD=2.45) and BMI of 21.5 kg/m^2 (SD = 1.10) participated. They were screened as in Experiment 1.

The cardiovascular response to arousal from sleep was measured under 2 conditions: threat and no threat. The conditions were run on separate, nonconsecutive, counter-balanced nights. In each condition, subjects were aroused from sleep by auditory stimuli selected from the battery of International Affective Digitized Sounds. In 1 condition, the stimuli contained threatening material, as indicated by ratings high on arousability and unpleasantness. They included auditory depictions of rape, murder, and child beating. In the other condition, they were low on arousability and high on pleasantness and included seashore sounds, flowing water, and gentle music. Ten scripts were used in each condition and were presented in random order for as many trials as could be obtained.

The University of Melbourne’s Human Ethics Committee approved the studies, and participants gave written informed consent and were reimbursed for their participation.

**Procedures**

The laboratory procedures were the same as for the previous experiments, with the exception that each arousal was produced by auditory stimuli, rather than being spontaneous, and the stimuli were presented during stage 2 sleep, rather than stage 1 or stage 2 sleep. Finally, a questionnaire was administered at the completion of data collection; this questionnaire assessed the participants’ subjective assessment of the arousing quality, and loudness of the stimuli on 10-centimeter visual-analog scales.

The stimuli were 6 seconds in duration and were delivered by computer via 2 speakers positioned on either side of the bed at the level of the subject’s head. They were presented following a minimum of 30 seconds of stage 2 sleep. In order that the influence of the physical intensity of the stimuli were minimized, the intensity was maintained so as to “just arouse” the subject. However, the intensity of a stimulus necessary to arouse an individual varies with the depth of sleep. Thus the physical intensity of the stimuli was varied using a staircase procedure so as to track sleep depth. Stimulus intensity was increased by 5 decibels (dB) if 2 consecutive presentations did not produce an arousal and decreased by 5 dB if 2 consecutive presentations both produced an arousal. (While the stimuli could be varied in 5-dB increments, because of the complexity of the stimuli, it was not possible to define their absolute intensity.) The stimulus intensity required to produce an arousal did not differ significantly between conditions (the arbitrary scale units were 2.9 and 3.9 for the nonthreat and threat stimuli, respectively (t[7]=1.60, P>.05).

**Data Reduction and Analysis**

An arousal was defined according to the ASDA criteria, with the additional requirement that the arousal had to occur within 2 seconds of the onset of the stimulus, although if an arousal was to occur, it typically began immediately upon stimulus onset. The data-analysis procedures were the same as in the previous experiments, with the exception that trials were aligned to the onset of the auditory stimulus. (It should be noted that the results were essentially the same if responses were aligned to the onset of the arousal.) The number of stimuli presented varied from 36 to 60 over nights, with a mean of 49, while the number of suitable ASDA arousal responses obtained varied over nights from 20 to 39, with a mean of 29. A mixed 2-by-2 ANOVA model was used with the 2 conditions, which were run on different nights, being treated as an independent factor and the preexperiment and postexperiment comparison as a repeated measures factor. t tests were used to compare scores on the postexperience questionnaire.

**RESULTS**

The results were identical to Experiment 2A. As indicated in Table 4 (and illustrated for HR in Figure 3, bottom panel), being aroused by a threatening stimulus did not increase the magnitude of the cardiovascular response at an arousal.

The questionnaire data indicated that subjects’ perception of their arousal frequency (4.5 arousals per night) was substantially less than the number of ASDA arousals elicited by stimuli (29 arousals per night if arousals with movement artifact are considered), although the subjective impression of the number did not differ between conditions (4.6 vs. 4.4 for threat and nonthreat conditions, respectively, t[7]=0.20, P>.05). However, subjects rated the stimuli on the threat night as more unpleasant (7.02 vs. 3.37, t[7]=5.16, P<.05) and arousing (6.07 vs. 4.19, t[7]=2.93, P<.05) but not different with respect to loudness (6.07 vs. 5.90, t[7]=0.26, P>.05).

**DISCUSSION**

Figure 3 indicates several differences in the HR response profile between the 2 experiments. Consistent with earlier studies (eg, Trinder et al) the onset of the response to a spontaneous arousal occurred earlier and was less clearly defined (Experiment 2A, top panel). This most likely reflects the poorer precision with which a spontaneous arousal can be identified as compared to one that follows an arousing stimulus. The earlier onset may also indicate that, in a spontaneous arousal, activation of the heart precedes that of the cortex. There was also some sugges-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Prearousal</th>
<th>Postarousal Mean</th>
<th>Postarousal Peak</th>
<th>Effect</th>
<th>F ratios (df=1,14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, bpm</td>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td>Mean</td>
</tr>
<tr>
<td>NT, TR</td>
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<td>62.8 (4.6)</td>
<td>69.6 (5.3)</td>
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<td></td>
<td>58.7 (6.2)</td>
<td>62.5 (5.6)</td>
<td>69.1 (4.8)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>P-P</td>
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<td></td>
<td>X</td>
<td>71.25**</td>
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<td></td>
<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>PV, au</td>
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<td>9.36 (2.8)</td>
<td>5.77 (1.2)</td>
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<td>9.36 (2.8)</td>
<td>6.56 (1.8)</td>
<td>3.39 (0.80)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td>71.34**</td>
</tr>
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<td></td>
<td></td>
<td>P-P</td>
<td>109.84**</td>
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<td></td>
<td></td>
<td>X</td>
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<td>0.05</td>
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<tr>
<td>SBP, mmHg</td>
<td>81.8 (9.3)</td>
<td>82.7 (5.6)</td>
<td>99.9 (12.8)</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>82.7 (5.6)</td>
<td>88.3 (4.3)</td>
<td>100.7 (6.0)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td>9.78**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P-P</td>
<td>49.34**</td>
</tr>
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<td></td>
<td></td>
<td>X</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.00</td>
</tr>
</tbody>
</table>

NT, nonthreat; TR, threat. HR, heart rate; bpm, beats per minute; PV, pulse volume; au, arbitrary units; SBP, systolic blood pressure; mmHg, millimeters of mercury; (*P<.05; **P<.01).
ton that HR did not return to the prearousal level following an arousal produced by a complex stimulus (bottom panel).

An observation of considerable interest was the bradycardia response on the first and second postauditory-stimulus heartbeats (bottom panel). This suggests an OR to the onset of the complex auditory stimuli. While posthoc analyses indicated that the combined subject effect was not significant, 6 of the 8 subjects showed significant falls in HR under 1 or the other condition in the first 2 poststimulus breaths. This was consistent with the large individual differences in the occurrence of the OR, reported in the literature. However, the size of the bradycardia component did not differ between conditions, whereas in studies conducted during normal wakefulness, aversive stimuli have produced more pronounced bradycardia. This suggests that the valence of the stimulus conducted during normal wakefulness, aversive stimuli have produced more pronounced bradycardia. This suggests that the valence of the stimulus when subjects were awake and the prearousal levels in the spontaneous arousal condition when subjects were asleep. In other words, the arousal appeared to return HR and BP to wakefulness levels. This interpretation is contrary to the proposal that the activation response anticipates, rather than depends upon, potential threats in the environment.

There are, however, several caveats to these conclusions. First, the manipulations used in these studies may not have been significantly intense to produce an effect. In response to this, we would point out that the magnitude of other reflexes are responsive to a subject’s level of fearfulness, as measured by the FSS, and other studies that have identified differential psychophysioligic effects in response to the International Affective Digitized Sounds stimuli. Second, it could be argued that the experiments had insufficient power to contemplate rejecting the null hypothesis. On the other hand, in neither experiment was there any suggestion of a small effect that may have become significant with additional power. Third, the gender imbalance between the conditions in Experiment 2A may have masked group effects. However, contrary to this was the absence of any suggestion of gender differences in the magnitude of the WR in Experiments 1 and 2A (exploratory analyses). Fourth, the particular interventions employed may not be those to which the activation response is sensitive, a possibility that can only be resolved with further study.

**EXPERIMENT 3**

Experiment 1 raised the possibility that cardiovascular activation at an arousal was due to the return of wakefulness and waking levels of activity. This interpretation was based on the observation that the magnitude of the HR and BP responses to a spontaneous arousal was similar to the magnitude of the sleep-onset effect (the sleep-onset effect was assessed by the difference between the prestimulus levels for the OS and SS conditions when subjects were awake and the prearousal levels in the spontaneous arousal condition when subjects were asleep). In other words, the arousal appeared to return HR and BP to wakefulness levels. This interpretation is contrary to the proposal that the activation response is a reflex, triggered by the act of arousing.

These two hypotheses make different predictions with respect to the timecourse of the activation response. Specifically, the view that the increase in cardiovascular activity at an arousal is due to the return of wakefulness levels of activity predicts that the waning of the response will be dependent on the reinstatement of sleep. In contrast, the reflex hypothesis predicts that the response, once elicited, will proceed with a predictable timecourse, independent of the sleep-wake state. These predictions can be tested by determining the timecourse of the activation response, as a function of the duration of the period of wakefulness.

**METHODS**

**Subjects and Design**

This study consisted of a retrospective analysis of the HR response to spontaneous arousal from sleep in 3 previous data sets. It was necessary to combine several data sets in order to obtain a sufficient number of spontaneous arousals covering a range of arousal durations. It was judged appropriate to combine these particular data sets as the studies used essentially identical procedures. The 3 studies were Experiments 1 and 2A of this paper and a recently published experiment.

Briefly, in the recently published study, 1 woman and 3 men were studied for 2 nights in each of 2 conditions. In 1 of the conditions, subjects were maintained on positive pressure ventilation, while the other was a control condition. Only the data from the 2 control nights has been used in this analysis. The resulting sample consisted of 34 subjects (20 women; 14 men). The HR responses to spontaneous arousal from stages 1 and 2 were analyzed using the same procedures as in the current studies. An average of 44 spontaneous arousals were obtained for each subject.

In the present analysis, each arousal was allocated to a condition based on the arousal’s duration. There were 4 conditions: more than 3 to less than 6 seconds, more than 6 to less than 10 seconds, more than 10 to less than 15 seconds, and 15 or more seconds.

**Definition of an Arousal**

An arousal was defined by the presence of alpha activity in the O1-A2 EEG lead. The onset was identified by the first indication of a spontaneous arousal, such as alpha activity, a K complex, or eye movement. The termination of the arousal was defined as the onset of predominant theta activity that continued for more than 10 seconds. The duration was defined as the time from arousal onset to the arousal termination. In addition, at least 3 seconds of alpha had to occur, although inevitably alpha activity either defined the onset, occurred within a second or 2 of the auditory stimulus, or followed immediately after a K complex or eye movement. Arousals during which body movements occurred were discarded, although arousals during which an increase in submental EMG activity was identified, without interference on other channels, were retained. Further, in the 15-or-more-seconds condition, the arousal was retained if a body movement did not occur before, but did occur after, 20 seconds.

**Data Analysis**

The data were presented graphically over the 20 heartbeats before and after an arousal for each of the conditions in a manner identical to the earlier experiments. For statistical analyses, the data was reduced to 6 periods by averaging heartbeat values within the specified periods for each subject. The periods were the 20 prearousal heartbeats and the postarousal beats 1 to 3, 4 to 6, 7 to 10, 11 to 15, and 16 to 20. The resulting model was a 4-arousal-duration conditions by 6-periods ANOVA with repeated measures on both factors. (It was recognized that the units of measurement were time in one factor and heartbeats in the other. However a rescaling of the heartbeat data was not carried out as HR approximated 60 beats per minute.) Posthoc comparisons were then conducted between conditions within each period. Finally, to determine if there was any difference in the magnitude of the peak response between

<table>
<thead>
<tr>
<th>Condition (Arousal Duration)</th>
<th>Prearousal</th>
<th>Period Number of Postarousal Heartbeats</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 3</td>
<td></td>
<td>0 - 3</td>
</tr>
<tr>
<td>4 - 6</td>
<td></td>
<td>4 - 6</td>
</tr>
<tr>
<td>7 - 10</td>
<td></td>
<td>7 - 10</td>
</tr>
<tr>
<td>11 - 15</td>
<td></td>
<td>11 - 15</td>
</tr>
<tr>
<td>16 - 20</td>
<td></td>
<td>16 - 20</td>
</tr>
<tr>
<td>1. &gt;3 s - &lt;6 s</td>
<td>62.8 (9.6)</td>
<td>67.2 (10.1)</td>
</tr>
<tr>
<td>2. &gt;6 s - &lt;10 s</td>
<td>62.4 (9.4)</td>
<td>68.1 (9.4)</td>
</tr>
<tr>
<td>3. &gt;10 s - &lt;15 s</td>
<td>62.8 (10.0)</td>
<td>67.9 (10.1)</td>
</tr>
<tr>
<td>4. &gt;15 s</td>
<td>63.4 (9.3)</td>
<td>68.7 (9.6)</td>
</tr>
</tbody>
</table>

Significant Differences

None

Table 5—Mean (SD) heart rate during the prearousal period and at various periods postarousal as a function of arousal duration for Experiment 3. Significant differences are for contrasts between conditions within periods (P<.05).
GENERAL DISCUSSION

The HR response to an arousal, as a function of arousal duration, is presented in Table 5 and Figures 4 and 5. The data indicated the presence of a transient activation response that was not dependent on when the subject returned to sleep. Thus, the response profile over the first 8 heartbeats was similar over the 4 conditions and, in the 3 conditions with the longest arousals, HR began to fall, with approximately the same trajectory, before subjects returned to sleep.

Nevertheless, the data also indicated that the response was influenced by the duration of the arousal. Statistically this was indicated by a significant conditions by periods interaction effect ($F_{[15,480]}=9.08, P<.001$). Further, the magnitude of the response at postarousal heartbeat 3 differed significantly over conditions ($F_{[3,96]}=3.89, P<.05$), as did the effect of arousal duration (there were significant differences between groups in periods 4 to 6, 7 to 10, 11 to 15, and 16 to 20—see Table 5).

Inspection of the data in Figures 4 and 5 suggested there were 3 processes operating. The first was the transient activation response, the recruitment phase being apparent over heartbeats 2 to 3. The second was an effect of arousal intensity, a more intense arousal having a larger peak response, a delay in the waning phase of the response, and a longer period of sleep. The third was an effect of the return of sleep. The presence of the third component was suggested by a comparison of the 4 conditions over the waning phase of HR. Thus, in the 2 conditions with relatively brief arousal periods, the falling phase of the transient activation response coincided with a return of sleep, the additive effects resulting in a reduction in HR below the prearousal level. In the more than 10 to less than 15 seconds condition, sleep had not resumed at the completion of the transient activation response, and the final fall in HR was delayed until it occurred. A similar effect was observed in the more than 15 seconds condition, in which the secondary fall in HR was delayed while the subjects were still awake. The overall higher HR in this condition was most likely due to greater arousal intensity.

RESULTS AND DISCUSSION

Consistent with recent studies, the present series of experiments identified a stereotyped cardiovascular activation response that occurred at both spontaneous and tone-elicited arousals from sleep. In the first experiment, the response was shown to have a strong cardiovascular response pattern and weak GSR and eye-blink startle response, distinguishing it from both the OR and SR. This suggested it was a different response to these 2 classic reflexes and should perhaps be separately designated. The term *waking reflex* has been suggested, although it has not been established whether the response is a true reflex.

Experiment 1 led to the investigation of 2 subsequent issues. First, the strong cardiovascular component was consistent with the view that the function of the response is to reinnate wakefulness levels of functioning in the event that the environment is threatening. However, Experiments 2A and 2B failed to provide supportive evidence for the original statement of the adaptive hypothesis, as the data showed that the morphology and magnitude of the response was independent of the psychological trait of fearfulness and of the nature of the threat valance of the arousing stimulus. An alternative interpretation of this finding is that the activation response is elicited by the act of waking and is independent of current environmental circumstances. According to this view, variations in the environment would not be reflected in cardiovascular functioning until after the environment had been surveyed, a process that would be preceded, and facilitated, by the waking activation response.

Second, in Experiment 1 it was observed that for the cardiovascular variables, the magnitude of the difference between wakefulness (baseline values for the OS and RS conditions) and sleep (prearousal values in the spontaneous-arousal condition) was very similar to the magnitude of the arousal response. This suggested that the cardiovascular activation at an arousal, rather than being a transitory arousal response, was a return to wakefulness levels of activity. Experiment 3 indicated the presence of a transient activation response that was not dependent on when the subject returned to sleep, thus the response profile over the first 8 heartbeats was similar over the 4 conditions and, in the 3 conditions with the longest arousals, HR began to fall, with approximately the same trajectory, before subjects returned to sleep.
ence of a transitory activation component as the HR response was not dependent on arousal duration. In particular, for longer arousals, HR began to fall toward prearousal levels even though the subject remained awake. Nevertheless, arousal duration did have some effect on the amplitude and duration of the response, and the fall in HR to prearousal, or lower, levels was dependent on the subject returning to sleep.

It is likely that the duration of an arousal reflects its intensity. If so, the data suggested that the magnitude (both peak response and duration) of the WR is influenced by the intensity of the arousal. Further, the data suggested a component of the response was attributable to the subject being awake, as distinct from the act of arousing. This observation has been reported previously in dogs, although it is inconsistent with a previous report that failed to observe changes in HR as a function of the transition from EEG alpha activity (wakefulness) to EEG theta activity (sleep).

In conclusion, in young healthy adults, an arousal from sleep, whether spontaneous or elicited by a stimulus, was associated with a vigorous cardiovascular response. The magnitude of the response was not directly affected by the properties of arousing stimuli or by characteristics of the aroused individual. Further, once triggered, the response had a predictable timecourse, although it’s magnitude and duration were influenced by either the duration or intensity of the arousal. Finally, there was a further component of the response that was attributable to the state of wakefulness, as distinct from the “arousal state.”

REFERENCES