INTRODUCTION

SLEEP DEPRIVATION RESEARCH HAS BEEN CARRIED OUT FOR A VARIETY OF REASONS. The earliest studies of sleep deprivation were undertaken in attempts to uncover the functions of sleep (i.e., how much sleep is necessary for survival). In the 1950s to the 1970s, an era of applied research was driven by military interest in human ergonomics (i.e., what is the minimum amount of sleep necessary to function optimally). Today, this research is often carried out with an interest in human health and safety, that is, researchers aim to understand the nature of central nervous system (CNS) impairment and the degree of performance deficit associated with sleep loss, particularly in sleep-disordered populations. The behavioral effects of total sleep deprivation on human performance, first reported more than 100 years ago, include prolonging of reaction time and deficits in motor ability, visual acuity, memory, and attention. Despite more than a century of study, researchers concur that there has been little progress in understanding the nature of these effects. Modern neurophysiologic methods such as quantitative electroencephalogram (EEG) analysis and event-related potentials (ERPs) offer promise for understanding performance deficits during varying levels of sleep loss through regional examination of brain functioning.

Quantitative analysis of the EEG, such as power spectral analysis (PSA), allows for description of CNS changes that may be associated with specific types and degrees of performance deficit. A topographic analysis of EEG at multiple electrode sites is useful to assess the effects of sleep loss at specific brain regions. A series of studies from the University of Mexico has investigated waking PSA at multiple electrode sites during total sleep deprivation. The studies indicate that there is an increase in power for the high frequencies (i.e., theta, beta) during total sleep deprivation relative to baseline. This finding may represent increased effort to maintain wakefulness or to perform on task. Indeed, when PSA was conducted on the EEG recorded during actual performance, the findings were more robust. In support of these studies, a single hertz analysis indicated that power density values in the 6.25-Hz to 9.0-Hz range increased during total sleep deprivation. These increases in the theta/alpha range were correlated with increasing subjective fatigue. Later reports indicated that increases in the theta range following total sleep deprivation were localized to frontal regions. Again, the changes in EEG frequencies were correlated with increases in subjective sleepiness.

Event-related potentials are cerebral potentials recorded from scalp electrodes and consist of a series of negative and positive deflections that reflect information processing of a stimulus event. Examining changes in the amplitude and latency of these waveforms may be used to determine if performance deficits result from errors in initial encod-
ing of information, motor execution of response, or later cognitive processes involving memory and attention. The late components of the auditory ERP peak from 50 to 350+ milliseconds following the onset of a stimulus. They are labeled P1 (positive peak at 50-75 milliseconds), N1 (negative peak at 80-100 milliseconds), P2 (positive peak at 175-225 milliseconds), N2 (negative peak at 250-350 milliseconds), and P300 (positive peak at 300+ milliseconds). These long-latency ERP are particularly sensitive to changes in attention and arousal, as indicated by changes during the sleep-onset period.11 During total sleep deprivation, changes in ERPs have been reported to be associated with subjective fatigue,12 alertness during the Multiple Sleep Latency Test,13 and performance impairment.14,15 The N1 latency has been reported to increase during sleep deprivation, indicating a delay in initial sensory-encoding processes.16,17 In addition, P300 latency has been shown to be delayed following extended wakefulness.12,15 These delayed latencies, in conjunction with lengthened reaction times on performance tasks, suggest that total sleep deprivation impairs the ability to make perceptual discrimination. Furthermore, various studies have reported decreased P300 amplitude following total sleep deprivation, supporting a role for impaired allocation of attention during sleep deprivation.12,15

The EEG and ERP studies converge to suggest that the nature of the effects of total sleep deprivation on brain functioning are at least in part due to reduced arousal and impaired attention. But what of the neurophysiologic impact of partial sleep loss or disrupted sleep; that is, levels of sleepiness more commonly experienced today such as sleep restriction or sleep fragmentation? Partial sleep deprivation may occur, for example, when the sleeper chooses to truncate his or her sleep either by going to bed later or rising earlier than usual. Insufficient sleep, especially over an extended period, is a common problem today as many people are cutting down on sleep in an effort to get more out of their day. In the laboratory, one experimental method of partial sleep deprivation is continuous sleep restriction. Dinges et al employed a continuous sleep-restriction paradigm in which total sleep time was reduced by 33% below habitual amounts over a 1-week period.14 They reported a linear decline on various neurobehavioral tasks over the 7 days of restricted sleep. No electrophysiologic data were reported.

Sleep fragmentation is a type of sleep disruption that involves inducing arousals by administering intrusive auditory stimuli periodically throughout the night. It is intended to be a model for the type of frequent and periodic disruption experienced in common sleep disorders such as sleep-related breathing and movement disorders where the sleeper may be aroused several hundred times during the night. Experimental sleep fragmentation allows the investigator to parse apart effects due to fragmented sleep itself versus the effects related to hypoxemia associated with apnea or neurologic correlates of periodic limb movements. The auditory-stimulus parameters used to fragment sleep have been well researched in the earliest studies of sleep fragmentation.9 These studies have consistently shown that daytime sleepiness results from this degree of sleep loss, although evidence of performance impairment has been inconsistent.20-35 The failure to find consistent evidence of performance impairment is not surprising in light of the difficulties associated with repeated performance assessment (eg, learning, motivation, individual differences, and interactions with task difficulty, task specificity, and time of day).36

Research has shown that both total and partial sleep deprivation lead to deficits in sleepiness, mood, and cognitive performance. Sleep fragmentation is a subtle form of sleep disruption that results in similar behavioral effects. There have been few electrophysiologic studies of daytime functioning following sleep fragmentation. One recent study investigated processing of visual stimuli using ERPs following a single night of sleep fragmentation.35 Researchers observed reduced P300 amplitude at some frontocentral sites, reflecting a decrease in attention. In the present study, we employed the methods of sleep fragmentation to investigate brain functioning associated with daytime sleepiness. Specifically ERP and quantitative EEG analyses were investigated following experimentally induced sleep fragmentation in healthy good sleepers. Daytime performance and electrophysiology were assessed repeatedly during a 40-minute test battery administered every 2 hours beginning at 9:00 AM and ending at 7:00 PM. The study was a within-subjects design where the order of conditions was fixed as follows: adaptation, baseline, sleep fragmentation night 1, sleep fragmentation night 2, and recovery. We hypothesized that neurophysiologic deficits consistent with impaired attention would be apparent on measures of ERPs and quantitative EEG, perhaps even in the absence of performance deficits. A secondary purpose was to investigate possible cumulative effects or habituation on the second consecutive night of sleep fragmentation.

METHODS

Participants

Participants were recruited through advertisements at the university and public service announcements in the community. These advertisements invited healthy individuals who were between the ages of 25 and 45, right-handed, fluent in English, good sleepers, nonsmokers, and free from medication to contact the sleep laboratory for additional information. In an initial telephone interview, participants were screened and excluded if they were shift workers, consumed more than 3 caffeinated beverages per day, or had history of depression, head injury, heart disease, neurologic disease, or chronic pain. It was also a prerequisite that participants be available for the 4-day protocol. Twenty-eight participants were excluded based on these criteria, and suitable candidates were invited to attend an orientation session at the laboratory. Eight participants were excluded after attending the orientation session: 1 revealed a diagnosis of Tourette syndrome, 1 was unwilling to comply with the caffeine restriction during the study, 3 were no longer interested in the study, and 3 were unavailable to participate due to scheduling conflicts. One participant was excluded after the screening night due to respiratory events of nonclinical significance. One participant withdrew from the study following the first night of sleep fragmentation due to “distress over isolation of the lengthy paradigm.”

Eight participants (4 women; mean age = 33.25 years, SD = 6.50) completed the protocol. Of these, 3 were married or cohabitating, and 5 were single at the time of study. Most had some postsecondary education (4 with bachelor’s degrees, 1 with a college diploma, 2 university students, and 1 with high school complete). Their current employment status included 5 full-time workers, 1 part-time worker, and 2 full-time students. None worked nights or shifts. Their occupations included professional, technical or skilled worker, and government employee.

Procedure

During an orientation session, participants were given a tour of the sleep laboratory, and procedures of the study were outlined prior to signing consent. Hearing thresholds were then verified to be within normal range (ie, below 15 dB ISO at 500, 1000, 1500, and 2000 Hz in both ears). A set of questionnaires was administered to gather information on sleep history and habits, health, and mood.37-39 Those meeting inclusion criteria were asked to spend a night at the laboratory during which time their sleep was recorded by standard clinical polysomnographic procedures in order to screen for respiratory and movement-related sleep disorders. Of the 8 participants included in the final analysis, their average sleep efficiency was 90% and their average sleep-onset latency was 20.40 minutes on the screening/habituation night. Following the screening night, participants were instructed to maintain regular sleep-wake schedules from the approximate hours of 11:00 PM to 7:00 AM and to record their sleep-wake times and activities in a diary for the period prior to the sleep-restriction protocol in the laboratory (on average, 10.38 days). Participants wore wrist activity monitors (ActiTrak, by IM Systems Inc.) to verify compliance with these instructions. All participants adhered to the regular sleep-wake schedule.

Participants then began a 96-hour continuous protocol during which nighttime sleep was monitored and daytime performance was assessed.
in the laboratory. Night 1 served as a baseline night, during which participants were allowed to sleep uninterrupted from 11:00 PM to 7:00 AM. During nights 2 and 3, sleep was fragmented using auditory stimulation, following methods similar to those used by Philip, Stooohs, and Guilleninault.2 Stimuli were delivered to the right ear via an insert earphone throughout the entire night in all stages of sleep. Beginning 5 minutes after consolidated sleep onset, identified by the presence of K-complexes or spindles, a tone at 65 dB was delivered for 10 seconds. If no arousal was noted within 5 seconds following the tone, its intensity was increased by 10-dB increments up to a maximum of 115 dB. If no arousal was elicited by the highest intensity, it was repeated, and the experimenter then entered the room to arouse the sleeper (checking to ensure that the earpiece was still in place). Arousals were determined based on EEG criteria (ie, an increase in alpha frequency following stimulation). Thus, movement on the electromyography (EMG) channel or a behavioral response was not required. These are the same arousal criteria employed in the 6 most recent studies of experimental sleep fragmentation reviewed by Stepanski.29-35 Once an arousal was noted, the intensity was reset to its lowest level at 65 dB, and the procedure was repeated when the participant reentered any stage of sleep for 30 seconds. The same 2 experimenters were present during the sleep-fragmentation nights. Any wake time during the night exceeding 1 minute (eg, washroom trips or macroarousals) was added to the time allotted in bed for each participant. The amount of time in bed added on experimental nights 2 and 3 never exceeded 30 minutes. The fourth night of the protocol was a recovery night during which the participants were again permitted to sleep uninterrupted. The order of experimental conditions (baseline, sleep fragmentation night 1, sleep fragmentation night 2, and recovery) was fixed. This fixed-order design was implemented in order to assess the effects of continuous sleep restriction on daytime performance. 

During all experimental conditions, nighttime sleep was recorded from 6 scalp sites (F3, F4, C3, C4, O1, and O2 sites) using gold electrodes and referenced to the left mastoid. An average of A1 and A2 was calculated off-line for the reference. Right and left electrooculograms (EOG) were recorded from upper and lower positions on the outer canthus of the eye. A bipolar EMG was recorded from electrodes placed below the chin. A ground electrode was placed on the forehead. Signals were sampled at 200 Hz and digitized using a 12-bit A-D card. Filter settings were 0.5 to 70 Hz for EEG and EOG and 5.0 to 100 Hz for EMG. 

Presleep and postsleep questionnaires were administered each morning and night. These included 4 visual-analog mood scales (eg, calm-irritable), a 7-point scale for sleepiness (1=feel active and vital, 7=almost in reverie), a 7-point scale for fatigue (1=full of energy, 7=totally physically exhausted), and the PANAS mood scale.40 The PANAS mood scale consists of 10 words that describe negative feelings (eg, distressed) and 10 words that describe positive feelings (eg, interested). The participants were asked to assign a number to each word, indicating the extent to which the word described how they felt at that moment (1=very slightly or not at all; 5=extremely). Separate scores were calculated for a Positive Affect Score and a Negative Affect Score.

During waking hours, participants completed a performance assessment battery (PAB) at 9:00 AM, 11:00 AM, 1:00 PM, 3:00 PM, 5:00 PM, and 7:00 PM each day. During each session, a 19-channel montage was recorded using a 64-channel tin electrode cap (Neurosoft Inc). The purpose of this enhanced electrode placement montage was to investigate topographic changes in EEG and ERP associated with daytime performance. The daytime EEG montage included frontal-parietal (Fz, F3, F4, F7, F8, Fp1, Fp2), central-temporal (Cz, C3, C4, T7, T8), parietal, (Pz, P3, P4, P7, P8), and occipital (O1, O2) sites. The EEG and the reference were the same as those applied in the sleep montage. Ground was placed at AFz in the electrode cap. The cap was placed on participants at 8:00 AM and worn continuously until 8:00 PM on the same day. Signals were checked prior to each PAB session; however, poor signals could not always be resolved prior to recording and an excessive use of gel sometimes shorted-out neighboring electrode sites. This was particularly problematic for parietal, occipital, and temporal sites furthest from the vertex (gel would move down, especially with long hair). An extensive topographic representation of electrophysiologic signals could therefore not be reported due to the large amount of missing data in systematic locations.

The PAB was constructed and administered using the software program E-Prime (Psychology Software Tools, Inc.). A customized 40-minute battery was delivered on a personal computer located in the participant’s bedroom. The tests were administered in a fixed order as follows:

Profile of Mood States (POMS): Participants were asked to describe their mood “right now” on a 5-point scale for 30 items.41 The POMS was the only task administered on paper.

Alpha Attenuation Task (AAT): The AAT provides a quantitative measure of variation in physiologic sleepiness.42 The EEG was recorded during 12 alternating periods of eyes-open and eyes-closed conditions (6 of each lasting 30 seconds in duration, plus a 5-second buffer period to eliminate EOG artifact associated with opening and closing of the eyes). The purpose of this task was to assess changes in EEG frequencies during varying levels of sleepiness. The “alpha attenuation coefficient,” the ratio of alpha power for mean eyes-closed to mean eyes-open, has been shown to be lower in a sleep-deprived group.

Serial addition and subtraction task: This task provides information on speed and accuracy on a complex task requiring sustained attention. In the math task, 2 numeric digits flash briefly on the computer screen in succession followed by either a “+” or a “-” sign. Participants are required to add or subtract the 2 numbers accordingly and enter the last single digit of the answer. The total duration of this task was 5 minutes.

Reaction time (RT) task: This task requires sustained attention and rapid response to auditory stimuli. Participants were instructed to respond as quickly as possible to an auditory tone by pressing the “0” key on the keypad. The participant’s RT was displayed on the monitor as means of feedback on each trial. The interstimulus interval varied at random from 2 to 10 seconds, and the total duration of the task was 10 minutes.

Auditory discrimination task: Participants were presented with a train of frequently occurring standard stimuli with a randomized interstimulus interval between 1 and 2 seconds. At random times, the participant was presented with a shorter-duration target tone. In response to the target, the participant was required to press the “0” key on the keyboard. Standard tones of 1000 Hz (200 milliseconds) were presented on 80% of trials, whereas the shorter target tones (100 milliseconds) were presented on 20% of trials. This oddball paradigm allows for averaging of evoked brain potentials that represent information processing of the auditory stimulus event. The usual oddball paradigm requires discrimination between low- and high-pitch tone pips, whereas the paradigm employed herein required discrimination of tonal duration. This was thought to be a more difficult task, particularly while sleepy. The total duration of this task was 4 minutes.

Perception of performance: This 5-point scale was displayed to gain subjective reports on “how well you feel you performed on this session of tasks” (very poor to very good).

Stanford Sleepiness Scale (SSS): Participants were asked to report their alertness level on a 7-point scale, in which each successive number represents an increasing level of sleepiness.43

The PAB was demonstrated to participants during their initial orientation session. In addition, on the baseline night (first night of experimental protocol), participants were asked to arrive early in order to practice the PAB. The entire battery was practiced once, with 2 additional sessions of the math task and 1 repetition of the auditory discrimination task.

During the 96-hour in-laboratory protocol, all meals were provided. Breakfast was available at 7:30 AM. Lunch and dinner, provided at noon and 6:00 PM, were purchased by the experimenters from the cafeteria at the university in order to provide nutritionally balanced meals. No caffeinated beverages were permitted at any time during study. A snack was
offered each night at 9:00 PM. Participants were led to believe that the study was extended until 11:00 PM on the recovery day; however they were in fact dismissed after their 7:00 PM hour PAB session. This deception was intended to reduce end-of-experiment effects, that is, an increase in performance associated with the anticipation of completion of the study. Participants were then compensated and debriefed.

### Data Analyses

Power spectral analyses: Quantitative EEG analysis was carried out on electrophysiologic data recorded during the AAT. Prior to any analysis, data was rereferenced off-line to an average of A1 and A2 for all EEG channels. The EEG bands selected for analysis were as follows: 4.0 to 8.0 (theta), 8.0 to 10.0 (alpha1), and 10.0 to 12.0 (alpha2). The PSA was conducted on the EEG at F3, F4, C3, and C4 sites. Artifact due to eye blinks or movement was visually inspected and removed. Eye movement artifact was determined by examination of the EOG channels and frontal sites. The Fast Fourier Transform (FFT) values were calculated for every 5.12 section of data that was available after artifact rejection (spectra record length). Within each spectra record, 2.56-second FFT analyses were performed and averaged together. A Hanning window with a 75% overlap of spectral records was employed. The FFT values were log transformed prior to statistical analysis to normalize the data.

Event-related potentials: The ERPs were obtained from electrophysiologic data recorded during the auditory discrimination task for both the target and the nontarget trials separately. Trials containing EEG +/- 100 μV were automatically excluded from the analysis. Within-subject averages were obtained for each experimental condition (baseline, sleep fragmentation night 1, sleep fragmentation night 2, and recovery) and then averaged across participants to create a grand average target and the nontarget trials separately. Trials containing EEG +/- 100 μV were automatically excluded from the analysis. Within-subject averages were obtained for each experimental condition (baseline, sleep fragmentation night 1, sleep fragmentation night 2, and recovery) and then averaged across participants to create a grand average target response and a grand average nontarget response. The peaks of the within-subject averages were scored using ERPScore. The amplitude and latency of N1 were measured as the most negative peak between 80 and 120 milliseconds, whereas the average amplitude of the P300 wave was calculated between 300 and 650 milliseconds.

### Statistical Analyses

Time-of-day differences in alertness and performance were not examined because of the small sample size. Within each experimental condition (baseline, experimental sleep fragmentation night 1, experimental sleep fragmentation night 2, and recovery), data from the 6 PAB sessions (9:00 AM - 7:00 PM) were averaged together to provide a single daily value for the various measures. Repeated measures analysis of variance (ANOVA) was run to evaluate changes across experimental conditions in sleep parameters, performance, mood, EEG frequencies, and ERP components (N1, P300). Greenhouse-Geisser corrections were applied where appropriate. Follow-up paired t-tests were run where the omnibus F-test was significant. In addition, polynomial contrasts were examined for linear, quadratic, and cubic trends across experimental conditions. Because the order of experimental conditions was fixed to include baseline, fragmentation night 1, fragmentation night 2, and recovery, a priori hypotheses were made that quadratic functions would fit the data.

### Results

### Sleep Parameters and Fragmentation

Sleep was fragmented approximately 25 times per hour (ie, 1 arousal per 2 minutes) using intrusive auditory stimuli. Both the mean number of tones delivered (603.75 on night 1 and 656.63 on night 2) and the mean number of arousals induced (192.25 on night 1 and 210.50 on night 2) did not differ significantly between experimental sleep fragmentation nights 1 and 2. These results indicate that the experimenters did not systematically alter their methods of sleep fragmentation between nights, nor did the participants respond differently to the sleep-fragmentation challenge. The consistency in the auditory-stimulation routine allows reliable investigation of the cumulative effects of sleep fragmentation.

Sleep was scored in 30-second epochs according to standard scoring methods outlined by Rechtschaffen and Kales. Interrater reliability was established at 92%. Repeated measures ANOVAs were run to compare a number of sleep parameters across the 4 experimental conditions (see Table 1). Results indicated that the sleep-fragmentation procedures were successful at disrupting sleep without reducing total sleep time (TST). The TST (in minutes) for baseline, sleep fragmentation night 1, sleep fragmentation night 2, and recovery was 420.56, 441.63, 416.00, and 436.56, respectively (F<1). The TST minus stage 1 sleep was also assessed in order to account for possible increases in stage 1 sleep confounding a true estimate of TST, as proposed by Wesensten and colleagues. Although there was a significant quadratic function depicting that the minutes in stage 1 sleep increased during the first experimental night (F(1,7) = 6.35, P<.05), the adjusted TST (minus stage 1) still did not reach significance. The percentage of time spent in stage 3 sleep, as well as the latency to stage 3 sleep, were both significantly different across experimental conditions. Follow-up t-tests indicated that there

### Table 1—Sleep variables across conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th></th>
<th></th>
<th></th>
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<th>ANOVA F statistic</th>
<th>ANOVA P value, η²</th>
<th>Polynomial Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TST</strong></td>
<td>420.56 (51.79)</td>
<td>441.63 (21.45)</td>
<td>416.00 (94.31)</td>
<td>436.56 (19.41)</td>
<td>0.39</td>
<td>Quadratic trend P&lt;.05, η²=.48</td>
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<tr>
<td>TST minus ST1</td>
<td>387.69 (52.33)</td>
<td>391.94 (22.93)</td>
<td>380.44 (93.16)</td>
<td>406.13 (23.07)</td>
<td>0.33</td>
<td>Linear trend P=.07, Cubic trend P=.07</td>
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<tr>
<td>Minutes in ST1</td>
<td>32.88 (17.02)</td>
<td>49.69 (28.37)</td>
<td>35.56 (11.29)</td>
<td>30.44 (19.84)</td>
<td>2.45</td>
<td>Quadratic trend P=.05, η²=.35</td>
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</tr>
<tr>
<td>SLPEFF, %</td>
<td>87.13 (10.40)</td>
<td>90.63 (4.24)</td>
<td>93.13 (2.03)</td>
<td>91.00 (4.11)</td>
<td>2.39</td>
<td>Linear trend P=.05, η²=.52</td>
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<tr>
<td>AROUSALS</td>
<td>7.38 (9.62)</td>
<td>1.75 (2.76)</td>
<td>1.13 (1.13)</td>
<td>2.75 (2.82)</td>
<td>3.53</td>
<td>Quadratic trend P=.05, η²=.48</td>
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<tr>
<td>WASO</td>
<td>24.63 (19.38)</td>
<td>10.38 (15.60)</td>
<td>3.19 (3.78)</td>
<td>9.13 (10.62)</td>
<td>2.18</td>
<td>Linear trend P=.05, η²=.48</td>
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</tr>
<tr>
<td>SOL</td>
<td>16.31 (11.83)</td>
<td>13.63 (14.50)</td>
<td>8.00 (8.65)</td>
<td>12.88 (11.76)</td>
<td>0.94</td>
<td>Linear trend P=.05, η²=.48</td>
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</tr>
<tr>
<td>S2 Latency</td>
<td>27.00 (24.70)</td>
<td>19.75 (13.87)</td>
<td>11.57 (8.54)</td>
<td>38.50 (17.05)</td>
<td>1.88</td>
<td>Linear trend P=.05, η²=.48</td>
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<tr>
<td>S3 Latency</td>
<td>42.25 (23.14)</td>
<td>64.13 (26.91)</td>
<td>28.86 (11.13)</td>
<td>25.38 (17.02)</td>
<td>3.20</td>
<td>Linear trend P=.05, η²=.48</td>
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<tr>
<td>S4 Latency</td>
<td>63.75 (33.32)</td>
<td>90.50 (67.56)</td>
<td>73.29 (62.85)</td>
<td>55.50 (46.60)</td>
<td>0.95</td>
<td>Linear trend P=.05, η²=.48</td>
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<tr>
<td>REM Latency</td>
<td>125.94 (49.17)</td>
<td>116.38 (51.21)</td>
<td>68.44 (31.24)</td>
<td>101.56 (40.69)</td>
<td>6.45</td>
<td>Linear trend P=.05, η²=.48</td>
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<tr>
<td>PT_W</td>
<td>5.09 (8.22)</td>
<td>1.56 (2.47)</td>
<td>0.68 (0.77)</td>
<td>1.63 (2.28)</td>
<td>2.36</td>
<td>Linear trend P=.05, η²=.48</td>
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<td>PT_MT</td>
<td>4.53 (29.00)</td>
<td>4.46 (1.67)</td>
<td>4.66 (1.63)</td>
<td>4.48 (2.96)</td>
<td>0.25</td>
<td>Linear trend P=.05, η²=.48</td>
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<td>PT_ST1</td>
<td>7.85 (3.74)</td>
<td>11.10 (5.96)</td>
<td>9.15 (3.94)</td>
<td>6.94 (4.38)</td>
<td>2.27</td>
<td>Quadratic trend P=.01, η²=.64</td>
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<tr>
<td>PT_ST2</td>
<td>60.53 (8.29)</td>
<td>63.95 (10.47)</td>
<td>58.30 (8.73)</td>
<td>58.36 (10.19)</td>
<td>1.58</td>
<td>Linear trend P=.05, η²=.31</td>
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<tr>
<td>PT_ST3</td>
<td>9.38 (2.65)</td>
<td>6.15 (4.34)</td>
<td>6.30 (4.00)</td>
<td>9.63 (2.58)</td>
<td>3.07</td>
<td>Quadratic trend P=.06</td>
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<tr>
<td>PT_ST4</td>
<td>2.25 (2.40)</td>
<td>1.94 (2.21)</td>
<td>3.24 (3.75)</td>
<td>2.39 (1.97)</td>
<td>0.45</td>
<td>Linear trend P=.05, η²=.30</td>
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<tr>
<td>PT_SWS</td>
<td>11.63 (4.41)</td>
<td>8.09 (5.61)</td>
<td>9.54 (6.64)</td>
<td>12.01 (3.47)</td>
<td>1.59</td>
<td>Quadratic trend P=.06</td>
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<tr>
<td>PT_REM</td>
<td>20.00 (6.14)</td>
<td>16.89 (6.76)</td>
<td>23.00 (8.13)</td>
<td>22.69 (6.35)</td>
<td>3.04</td>
<td>Linear trend P=.05, η²=.30</td>
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</table>

Note: TST = total sleep time (min); ST1 = stage 1 sleep; ST2 = stage 2 sleep; ST3 = stage 3 sleep; ST4 = stage 4 sleep; REM = rapid eye movement; SLPEFF = sleep efficiency (TST/time in bed); AROUSALS = number of major arousals; WASO = minutes of wake after sleep onset; SOL = sleep-onset latency (min); Remaining values represent percentage of time spent (PT) in the respective sleep/wake stages and latency to onset of stage; W = wake; MT = movement time; SWS = slow wave sleep (stage 3 + stage 4)
was a trend for an increase in time spent in stage 3 sleep on the recovery night compared to both the first ($P=.07$) and the second night ($P=.07$) of sleep fragmentation. This finding is also reflected in a trend for a quadratic function ($P=.06$) depicting a change in the amount of stage 3 sleep across the 4 experimental conditions. Latency to stage 3 sleep was significantly longer on the first night of fragmentation relative to the second night of fragmentation ($P<.05$) and the recovery night ($P<.05$). This rebound effect, apparent in the abbreviated latency to slow wave sleep following fragmented nights, may also be seen in both rapid eye movement (REM) latency ($P<.05$) and the percentage of time in REM sleep ($P=.06$) on the second night of fragmentation compared to the first night of fragmentation. The REM-sleep latency was significantly abbreviated on the second night of fragmentation relative to both baseline ($P<.01$) and recovery nights ($P=.06$). Similarly, there was a trend for less time in REM sleep in the first fragmentation night, relative to both baseline ($P=.06$) and recovery nights ($P=.09$). There were no statistical differences across conditions on any of the other sleep-wake parameters examined (refer to Table 1 for means and SD).

**Behavioral Data**

Subjective reports of sleepiness, fatigue, and mood were assessed using presleep and postsleep questionnaires. Postsleep values were analyzed to investigate participants’ self-assessment following each of the 4 experimental conditions. Fatigue and sleepiness did not significantly differ across conditions ($F<1$), however, there was a linear increase in sleepiness over the duration of the experiment ($M_{1}=2.75, M_{2}=3.50, M_{3}=3.75, M_{4}=3.62; F(1,7)=6.72, P<.05, \eta^{2}=.49$). On visual-analog scales of mood, there were significant condition effects for the calm-irritable ($F(3,18)=5.18, P<.01, \eta^{2}=.46$) and the relaxed-tensed ($F(3,18)=5.47, P<.01, \eta^{2}=.48$) descriptors. Follow-up $t$-tests indicated that participants described greater irritability following fragmentation night 1 ($M=50.00, SD=20.26$) compared to baseline ($M=32.14, SD=16.72, t(7)=-2.22, P<.06$), fragmentation night 2 ($M=32.86, SD=24.86, t(7)=-.39, P<.05$) and recovery ($M=26.14, SD=19.75, t(6)=3.51, P<.01$). In addition, participants described significantly less “tension” on the recovery night ($M=19.86, SD=12.51$) compared to baseline ($M=33.43, SD=21.98, t(7)=2.22, P<.07$), fragmentation night 1 ($M=46.00, SD=24.93, t(6)=3.36, P<.05$), and fragmentation night 2 ($M=41.71, SD=25.28, t(6)=3.59, P<.01$). There was a significant quadratic polynomial contrast for the relaxed-tense ($F(1,6)=8.58, P<.05, \eta^{2}=.59$) descriptor and a trend for the calm-irritable ($F(1,6)=5.16, P=.06$) descriptor, indicating that participants were more tense following experimentally induced sleep-fragmentation conditions compared to baseline and recovery. There were no significant condition effects or contrasts for the happy-sad or energetic-sluggish mood descriptors. There was a significant condition effect for PANAS positive mood, ($F(3,21)=4.58, P<.01, \eta^{2}=.40$). Follow-up $t$-tests revealed that positive mood was greater at baseline ($M=24.88, SD =11.42$) than all other conditions ($M_{1}[SD]=17.38 [7.35], 19.00 [9.40],$ and 19.50 [9.61]). There was, however, also a significant quadratic polynomial contrast for PANAS positive mood ($F(1,7)=9.69, P<.05, \eta^{2}=.58$), indicating that mood was impaired following fragmented nights compared to baseline and recovery. The PANAS negative mood did not vary significantly across experimental conditions.

Subjective measures of mood, sleepiness, and performance on RT and math tasks were assessed during the daytime following each experimental condition. These measures were taken during a PAB administered 6 times each day, beginning at 9:00 AM and ending at 7:00 PM (ie, 2-hour intervals). The data from PAB sessions were averaged together to obtain a single daily value for each experimental condition. The POMS mood scale was administered at the beginning of each PAB session. There was a significant quadratic polynomial contrast for the Vigor-Activity subscale ($M_{1}[SD]=8.27 [4.33], 5.52 [3.76], 6.04 [4.23], 7.19 [5.51]$; $F(1,7)=5.97, P<.05, \eta^{2}=.46$). Other POMS subscales, including Depression-Dejection, Anger-Hostility, Confusion-Bewilderment, Tension-Anxiety, and Fatigue-Inertia, as well as the total POMS mood score, did not change significantly over experimental conditions. The SSS administered at the end of the PAB sessions differed significantly across conditions ($F(3,21)=3.00, P<.05, \eta^{2}=.30$). Paired $t$-tests
indicated that sleepiness during recovery (M [SD]=3.32 [1.57]) was significantly less than that following the second consecutive night of fragmentation (M [SD]=3.94 [0.87]; t(7)=2.62, P<.05). Means plots for mood and sleepiness are illustrated in Figure 1. There were no significant condition effects on the serial addition or subtraction math task for the following dependent measures: accuracy (percentage correct), number of errors, number correct, mean response time (milliseconds), and median response time (milliseconds). Performance on the 10-minute RT was investigated for condition effects using the following dependent measures: mean RT, median RT, SD of RT, number of lapses (RT > 500 milliseconds), 10% fastest RTs, and 1/ slowest 10% RTs. None of these measures of RT differed across conditions (F<1). Finally, perception of performance marked on a 5-point scale did not significantly differ across conditions (F<1).

Quantitative EEG

Each PAB included recording EEG during alternating periods of eyes opened and eyes closed. A full topographic analysis of EEG was not possible because of poor-quality signals that resulted from the lengthy protocol. The lost data tended to be at sites most distal from the vertex (eg, excessive electrode gel built up at temporal, parietal, and occipital sites). Spectral data from C3, C4, F3, and F4 sites were analyzed. Two participants were excluded from the analysis of EEG due to a large amount of missing data. The PSA was conducted on artifact-free epochs of the EEG during eyes opened and closed periods separately. An alpha:theta ratio was calculated as a measure of relative slowing of cortical EEG. Higher ratio values indicate greater alertness. One-way ANOVAs (Condition) were carried out on each site separately for the alpha:theta ratio (eyes closed). (Refer to Figure 2 for the log-transformed means of alpha:theta power across conditions.) Significant main effects for Condition were apparent for right-hemisphere sites at F4 (F(3,15)=3.58, P<.01, η²=.42) and C4 (F(3,15)=10.16, P<.05, η²=.67). Although the direction of means appeared similar for left-hemisphere sites, the strength of the effect was only a trend (P=.07 at F3, P=.08 at C3). Follow-up paired t-tests for the right frontal site (F4) indicate that the alpha:theta ratio was significantly lower following the second night of experimental sleep fragmentation compared to both the baseline (t(5)=2.79, P<.05) and the first night of fragmentation (t(5)=2.57, P<.05). Similarly, at the right central site (C4), the alpha:theta ratio was significantly lower following the second night of experimental sleep fragmentation compared to baseline (t(5)=5.37, P<.01), the first night of fragmentation (t(5)=3.47, P<.05), and recovery (t(5)=3.67, P<.01). In addition, baseline also differed from the first night of sleep fragmentation for the alpha:theta ratio at C4 (t(5)=2.70, P<.05). The alpha attenuation coefficient, the ratio of mean alpha power for eyes-closed to eyes-open, was investigated for changes across experimental conditions. There were no significant main effects across Condition (F<1). There were also no significant effects for alpha (total, high, or low frequency) or theta with eyes opened or closed (F<1).

Event-related Potentials

The PAB contained a 4-minute auditory discrimination task. Participants were required to identify a shorter duration tone as their target and respond by pressing the “0” on the keyboard. The N1 and P300 latency and amplitude were measured to assess the hypothesized changes in attention following experimentally induced sleep fragmentation relative to baseline and recovery conditions. Six participants were included in this analysis due to poor recordings for 2 participants. As with the behavioral data, data collected from all 5 daytime PAB sessions were averaged to produce a single value for each condition in order to increase the number of trials available for averaging.

Figure 3 depicts the N1 response to the target stimuli across all experimental conditions. No difference in N1 latency was found across experimental conditions (F<1). A 1-way repeated-measures ANOVA was run to test for a Site (3) x Condition (4) effect on N1 amplitude. There was a significant main effect for site, F(2)=24.24, P<.05, η²=.67. Although the direction of means differed across conditions (F<1). A Stimulus type (target, standard) by Condition (4) ANOVA showed that there was a significant main effect for stimulus type (F(1,4)=8.43, P<.05, η²=.69) verifying that the amplitude of target was larger than the standard stimulus. A Site (Fz, Cz, Pz) by Condition (4) ANOVA indicated a significant main effect for Site (F(2,8)=4.57, P<.05, η²=.53) indicating that P300 amplitude following the target stimulus was largest at Pz. There were no significant main effects for Condition (F=1).

DISCUSSION

These data represent an example in which TST was not reduced through the experimental sleep-fragmentation procedures. It is thus a very subtle form of sleep disruption, uncontaminated by reduced TST, in which to investigate the extent of daytime impairment on neurobehavioral tasks and brain physiology. There has been some debate concerning the interpretation
of sleep-fragmentation data. While some researchers maintain that sleep fragmentation leads to shortened sleep duration and increases in stage 1 sleep, others argue that it is the lack of sleep continuity or the fragmented nature of the sleep itself that leads to daytime consequences. More recent studies of sleep fragmentation employing EEG-based criteria to define arousals from sleep (eg, increases in alpha EEG) rather than awakenings or behavioral responses have shown daytime deficits when sleep architecture has remained in tact. The data in the present study support the position that the effects of sleep fragmentation result from a disruption to sleep continuity rather than a gross reduction in TST or an increase in light stage 1 sleep. In addition, both REM and slow-wave sleep were affected by the experimental sleep-fragmentation procedures, indicating that the paradigm was not a stage-specific sleep-deprivation experiment.

Relative to baseline and recovery sleep conditions, participants reported disturbed mood and increased sleepiness during the days following sleep fragmentation. In support of this, quantitative EEG analysis indicated less cortical activation (alertness) that was cumulative across the 2 consecutive nights of sleep fragmentation. There was no performance deficit on simple tasks involving RT and mathemat processi...
41. McNair, D. Educational & Industrial Test Services; 1971.