

# Neural basis of alertness and cognitive performance impairments during sleepiness. I. Effects of 24 h of sleep deprivation on waking human regional brain activity

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Accepted in revised form 27 June 2000; received 23 November 1999

**SUMMARY** The negative effects of sleep deprivation on alertness and cognitive performance suggest decreases in brain activity and function, primarily in the thalamus, a subcortical structure involved in alertness and attention, and in the prefrontal cortex, a region subserving alertness, attention, and higher-order cognitive processes. To test this hypothesis, 17 normal subjects were scanned for quantifiable brain activity changes during 85 h of sleep deprivation using positron emission tomography (PET) and <sup>18</sup>Fluorine-2-deoxyglucose (<sup>18</sup>FDG), a marker for regional cerebral metabolic rate for glucose (CMRglu) and neuronal synaptic activity. Subjects were scanned prior to and at 24-h intervals during the sleep deprivation period, for a total of four scans per subject. During each 30 min <sup>18</sup>FDG uptake, subjects performed a sleep deprivation-sensitive Serial Addition/Subtraction task. Polysomnographic monitoring confirmed that subjects were awake. Twenty-four hours of sleep deprivation, reported here, resulted in a significant decrease in global CMRglu, and significant decreases in absolute regional CMRglu in several cortical and subcortical structures. No areas of the brain evidenced a significant increase in absolute regional CMRglu. Significant decreases in relative regional CMRglu, reflecting regional brain reductions greater than the global decrease, occurred predominantly in the thalamus and prefrontal and posterior parietal cortices. Alertness and cognitive performance declined in association with these brain deactivations. This study provides evidence that short-term sleep deprivation produces global decreases in brain activity, with larger reductions in activity in the distributed cortico-thalamic network mediating attention and higher-order cognitive processes, and is complementary to studies demonstrating deactivation of these cortical regions during NREM and REM sleep.

**KEYWORDS** alertness, cognitive performance, prefrontal cortex, regional brain activity, sleep deprivation, thalamus

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## INTRODUCTION

Lack of adequate sleep, or sleep deprivation, reduces workplace productivity, public safety, and personal well being (Dement and Vaughan 1999). Sleep deprivation is one cause of

accidents and catastrophic failures in real-world situations (Mittler *et al.* 1988), including military friendly fire incidents (Belenky *et al.* 1994) and vehicular accidents (Horne and Reyner 1995). Short periods of total sleep deprivation (i.e. 24 h) typically occur in instances where individuals or groups undergo extended wakefulness to meet deadlines. Longer periods of sleep deprivation (i.e. greater than 40 h) can occur during atypical sustained work conditions (Kroemer *et al.* 1990), such as in some military training exercises and combat operation missions (Haslam 1982; Krueger 1991; Belenky *et al.* 1994) and civilian emergency work situations (Krueger 1989). Substantial sleep deprivation can also occur in individuals suffering from sleep disorders (Kelly 1991), in those with suspected neurologic dysfunction (Williams *et al.* 1996), and in the elderly (Reynolds *et al.* 1987).

Two cardinal features of sleep deprivation are diminished alertness and cognitive performance. These neurobehavioral deficits are well established, beginning with the first published study of long-term human sleep deprivation over 100 years ago (Patrick and Gilbert 1896). Reduced alertness has been shown in short- as well as long-term sleep deprivation studies using objective and/or subjective measures of sleepiness (e.g. Carskadon and Dement 1979; Mikulincer *et al.* 1989; Newhouse *et al.* 1989; Penetar *et al.* 1993; Harma *et al.* 1998). Decrements in cognitive performance, often independent of loss of alertness or lapses in attention, are also produced by both short- and long-term sleep deprivation. Simple task performance is impaired, as reflected by tests of reaction time, vigilance, and attention (e.g. Horne 1988a; Dinges and Kribbs 1991; Koslowsky and Babkoff 1992; Gillberg and Akerstedt 1998). Similarly, complex task performance is impaired, as reflected by tests of working memory, verbal fluency and speech articulation, language, logical reasoning, creative and flexible thinking and planning, decision making, and judgment (e.g. Banderet *et al.* 1981; Horne 1988a; Newhouse *et al.* 1989; Harrison and Horne 1997, 1998, 1999). Performance deficits can occur as early as during the first night without sleep (Angus and Heslegrave 1985; Monk and Carrier 1997) and are amplified after two-to-three nights without sleep (e.g. Horne and Pettit 1985; Koslowsky and Babkoff 1992; How *et al.* 1994).

The degrading effects of sleep deprivation on alertness and cognitive performance suggest alterations in underlying brain physiology and function. To date, however, only a few studies have investigated *in vivo* brain activity changes mediating sleep deprivation-induced neurobehavioral impairment in normal volunteers. In the first study, Wu *et al.* (1991) quantified absolute changes in regional cerebral glucose metabolic rate (CMRglu), a marker for neuronal activity, using <sup>18</sup>Fluorine-2-deoxyglucose (<sup>18</sup>FDG) (Reivich *et al.* 1979) and positron emission tomography (PET) (Cherry and Phelps 1996) during a Continuous Performance Test, a visual vigilance task. At 32 h of sleep deprivation, significant decreases in absolute regional CMRglu were found in thalamus and cerebellum along with significant decreases in relative regional CMRglu (absolute regional CMRglu normalized to the whole brain) in these same regions and temporal cortex. These brain deactivations were

accompanied by a concomitant decrease in task performance. In another study, Drummond *et al.* (1999a, 2000) evaluated alterations in the cerebral hemodynamic response using blood-oxygen-level-dependent functional magnetic resonance imaging (BOLD-fMRI) during both a Serial Subtraction task and a Verbal Learning task. The Serial Subtraction task, a variant of the Serial Addition/Subtraction task (Thorne *et al.* 1985), involved attention, working memory, and arithmetic subtraction, while the Verbal Learning task involved recognition and recall. Statistical comparisons between normal wakefulness and 35 h of sleep deprivation revealed decreased BOLD responses, associated with impaired arithmetic performance, in the prefrontal anterior cingulate gyrus, lateral posterior parietal lobules, pulvinar thalamus, and visual cortices (Drummond *et al.* 1999a). With impaired verbal recall performance (Drummond *et al.* 2000), decreased BOLD responses were found in the prefrontal anterior cingulate and temporal lobes, and increased BOLD responses were noted in both the prefrontal and lateral posterior parietal regions.

Although the Wu *et al.* (1991) study provided the first quantitative assessment of absolute human brain activity changes and cognitive function during extended wakefulness, longer periods of sleep deprivation beyond 32 h, i.e. the effects of more than one night of sleep deprivation, were not evaluated. Moreover, the regions of interest analysis used (e.g. single activity measure over an entire cortical lobe) was not as sensitive as the more recent voxel-based method of analysis (e.g. statistical parametric mapping [SPM]; Friston *et al.* 1995a,b). This latter analysis allows assessment of multiple, smaller functional areas of cortex. Either of these factors may have minimized other significant regional effects of sleep deprivation.

The Drummond *et al.* (1999a, 2000) sleep deprivation findings were based on the BOLD-fMRI technique that evaluates the hemodynamic response to neuronal activation using high spatial resolution scanning. Because the technique does not utilize radiotracers and long scanning periods, several tasks can be evaluated in an experimental session. While a sensitive indicator of relative cerebral activation, the BOLD signal as currently applied is not a quantifiable measure of neural activity (Howseman and Bowtell 1999). Relative changes in the regional hemodynamic response are obtained by comparing the BOLD signal during the task of interest to the BOLD signal during a baseline or control task (Ogawa *et al.* 1998). The ability to quantify absolute brain activity in investigations of sleep deprivation may be important, however, when global activity is affected, as changes based on relative brain activity alone cannot characterize with certainty the magnitude or the direction of changes in regional brain activity response. On the other hand, when absolute quantification of brain activity has been accomplished and a whole brain or global change has occurred, the normalization procedure (e.g. transforming the data to *z* scores or ratios, or removing the global effect with analysis of covariance [ANCOVA]), can exclude some regions in terms of statistical significance. Also, other regions may appear activated or deactivated,

depending on the direction of the global change, when in fact a real change has not occurred (see Braun *et al.* 1997 and Kajimura *et al.* 1999 for related discussions). These aspects of brain imaging data acquisition and analysis – lack of absolute quantification and the statistical analysis of normalized (i.e. relative) values without concomitant analysis of absolute values – may obscure the extent and/or interpretation of regional brain activity changes.

In the present study, we quantified absolute regional CMRglu with  $^{18}\text{F}$ FDG and PET four times each in 17 normal volunteers during 85 h of sleep deprivation and utilized the SPM method for analysis of the absolute and relative neuroimaging data. During the four-day experimental phase, subjects were scanned after a night of normal sleep and then serially after 24, 48, and 72 h without sleep. During each  $^{18}\text{F}$ FDG uptake, and at 2-h intervals between PET scans (Fig. 1), subjects performed a computer-based Serial Addition/Subtraction task (Thorne *et al.* 1985). As shown in Fig. 1 and by other sleep deprivation studies (e.g. Thorne *et al.* 1983; Newhouse *et al.* 1989; Penetar *et al.* 1994; Drummond *et al.* 1999a), Serial Addition/Subtraction is sensitive to the effects of sleep deprivation, even when performed for short durations. This task is more complex than tests of simple reaction time or vigilance and involves not only sustained attention, but also working memory and arithmetic processing. All of these mental processes have been attributed, in large part, to the prefrontal cortex (e.g. Cohen *et al.* 1988; Coull *et al.* 1996, 1998; Dolan *et al.* 1997; Roland and Friberg 1985; Dohaene *et al.* 1996; Dehaene *et al.* 1999).

The main purpose of our experiment was to quantify and characterize global and regional brain activity changes implicated in sleep deprivation-induced neurobehavioral impairment during cumulative, extended sleep loss. We endeavored to model real-world sustained operations requiring wakeful-

ness and near continuous task performance across four consecutive days. Based on previous behavioral research, we expected ‘dose-dependent’ decreases in alertness and cognitive performance with cumulative sleep deprivation. We hypothesized that sleep deprivation would result in dose-dependent deactivation of the thalamus, a subcortical structure involved in alertness and attention (Mesulam 1985). Additionally, we hypothesized that sleep deprivation would produce dose-dependent deactivation of the prefrontal cortex, a region that subserves higher order cognitive processes (Fuster 1989; Frith and Dolan 1996) along with alertness and attention (Posner and Tudela 1997). Prefrontal cortical vulnerability to sleep deprivation has been suggested previously by Horne (1988b, 1993). We extended our prefrontal cortical deactivation hypothesis during sleep deprivation to include the anterior cingulate gyrus because of its participation in attentional processes (Vogt *et al.* 1992).

This is the first of a series of reports examining progressive changes in regional CMRglu with increasing sleep deprivation. We describe here the results of 24 h of sleep deprivation compared to rested baseline. These results have been published previously in preliminary form (Thomas *et al.* 1998a,b).

## METHOD

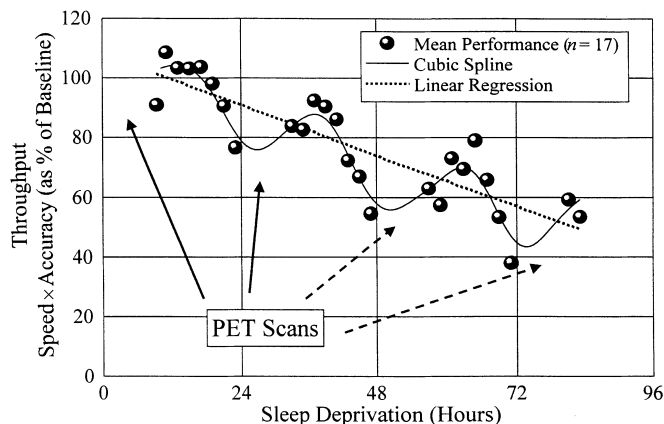
### Subjects

The 17 volunteers participating in the study were right-handed civilian males, between the ages of 21–29 years (mean  $24.7 \pm 2.8$  years), with no history of medical, neurological, psychiatric, or sleep disorder conditions. Their histories also included 7–8 h of nightly sleep on a regular basis, no nicotine use, and low caffeine use (less than 100 mg/day). Subjects passed a physical examination, including CBC and electrocardiography (EKG) tests and a narcotics screening test. Subjects were within normal range on mental states exams (Beck Depression Inventory, Beck *et al.* 1961; and Leeds Anxiety-Depression Scales, Snaith *et al.* 1976) and a cognitive test (Wonderlic® Personnel Test, Wonderlic, Inc., Libertyville, Illinois, USA).

Informed, written consent was obtained from all subjects. Subjects were paid for their participation in the study.

### Experimental design and methodological considerations

A time series design was used, with progressive sleep deprivation as the independent variable. Repeated measures of absolute regional CMRglu, cognitive performance, alertness, mood, and subjective experiences were collected after 0, 24, 48, and 72 h of sleep deprivation. Additional measures of alertness, cognitive performance, and mood were collected at fixed intervals throughout the sleep deprivation period. These measures were included to place the performance results associated with the PET scans in the context of the circadian rhythm of cognitive performance, as well as to impose a moderate-to-heavy near continuous workload on the subjects as might be anticipated in a real-world sustained operation.



**Figure 1.** Graph of the cognitive performance decline, modulated by the circadian rhythm, for the Serial Addition/Subtraction task during the 85 h of sleep deprivation. Data points are associated with performance measurements collected approximately every 2 h between PET scans, where Serial Addition/Subtraction task duration was 2–3 min. Hatched arrows indicate temporal occurrence of long-term sleep deprivation  $^{18}\text{F}$ FDG-PET scans, which will be reported separately.

Ideally, the evaluation of a rested control group of subjects, for whom nightly sleep occurred for three days in place of sleep deprivation, would have been helpful to account for potential nonspecific effects on brain activity (e.g. regional CMRglu effects that may be produced by task habituation, by day-to-day variability in regional brain activity, by unbalanced order of scans, or by learning and/or task tedium effects). Additionally, a sleep deprived control group, in which a performance deficit did not occur, may have been useful to possibly delineate primary or first-order effects of sleepiness on regional brain activity; e.g. perhaps indicating just one or two brain areas directly affected by sleepiness and therefore responsible, via their connectivity, for remote areas of deactivation. Also of interest would have been the addition of a fifth PET scan at the end of the study to assess recovery sleep effects on brain activity and task performance as well as to have compared several tasks within the same study to address the issue of task specificity and regional brain activity response to sleep deprivation.

In our four-consecutive day  $^{18}\text{F}$ FDG-PET scanning study, which included a pre-experiment day where subjects underwent a simulation PET scan, the addition of an extra PET scan to assess recovery sleep effects on subsequent wakefulness and the addition of a rested control group were precluded because of cost and logistical constraints. Given this circumstance, the comparison of the sleep deprivation scans to the baseline rested scans was a reasonable alternative to using a rested control group. For similar reasons, we were not able to include a sleep deprived-control group where performance did not vary. Even so, stable performance levels, in terms of both accuracy and reaction times, would have been impractical to accomplish out to 72 h of sleep deprivation, without either changing the task itself (i.e. making it considerably easier) or adding further financial incentives. Implementing these manipulations may not have proven successful, though, as in a previous study where substantial monetary rewards were given for maintaining performance at rested baseline values, intact performance could not be achieved on a simple vigilance test at 48–72 h of sleep deprivation (Horne and Pettitt 1985). Consequently, we focused our investigation on the underlying brain physiology of performance deficit, rather than intact performance, because we were most interested in delineating the regional brain pattern associated with the behavioral impairments. The use in our study of two additional days of sleep deprivation was a viable approach to discerning brain areas that might be more sensitive (i.e. show greater deactivation than other regions) to sleep deprivation. With respect to the effect of the specific task and brain activity response to sleep deprivation, a radiotracer with quantification capability, a very short half life, and low radiation exposure would have been required to allow evaluation of absolute brain activity responses during multiple task performances within a brief time window. The tracer  $\text{H}_2^{15}\text{O}$ , which measures cerebral blood flow (a correlate of cerebral glucose metabolism) and allows up to 12 PET scans per subject, meets these criteria. However, because of its short half life, quantifying absolute blood flow

by the  $\text{H}_2^{15}\text{O}$ -PET method necessitates automatic arterial sampling. Inserting an arterial line in the same subject in his one available wrist on four contiguous days would have been technically unfeasible in our study.

We did attempt within our experimental design to minimize scan order and other nonspecific effects. Firstly, we included a realistic simulation (except for injection of actual radioisotope) of the  $^{18}\text{F}$ FDG uptake and PET scan procedure prior to the first experimental PET scan. This was done to avoid possible novelty, anxiety, or excitement effects due to the introduction of the imaging procedure (Roland 1993) during the rested baseline scan. Subjective measures of tension and calmness showed that anxiety and excitement levels were not significantly different between the rested baseline and 24 h sleep deprivation scans (see Results). Secondly, we tested our subjects during performance of the same complex cognitive task in the four  $^{18}\text{F}$ FDG uptake periods. It has been shown in test-retest neuroimaging studies that the use of a standard task (vs. a 'rest' task) reduces inherent between-subject and day-to-day regional CMRglu variability (Duara *et al.* 1987; Holcomb *et al.* 1993). Thirdly, we included training and practice on the Serial Addition/Subtraction task prior to the baseline PET scan to preclude the situation of comparing learning and novelty effects of the task at the baseline scan vs. practice effects at the 24 h, and subsequent, sleep deprivation scans. Finally, we gave feedback of performance to subjects at 5-min intervals throughout the 30 min Serial Addition/Subtraction task and  $^{18}\text{F}$ FDG uptake to assist in sustaining effort and motivation levels (Wilkinson 1961). With sleep deprivation, we observed a significant increase in subjectively rated effort and a trend for increased motivation to perform the task (see Results) indicating that the cognitive performance deficits were most likely due to a direct effect of sleep deprivation on attention and cognition and not to an indirect effect of decreased effort and motivation produced by task repetition- or task duration-induced tedium.

## Procedures

### *Pre-study phase*

Each volunteer wore a Precision Control Design (PCD Inc., Fort Walton Beach, Florida, USA) BMA-32 wrist-worn movement activity device or actigraph (Redmond and Hegge 1985), 7–10 days prior to entering the study to document his adherence to a 22.00 to 05.45 h nightly sleep schedule, the sleep schedule prescribed in the nighttime sleep portion of the study. Subjects were advised to refrain from caffeine intake for the three days before the start of the study.

### *Acclimation and training phase*

Each in-residence session lasted eight days. Subjects arrived on Day 1 in groups of three or four at the Division of Neuropsychiatry, WRAIR. Their pre-study actigraph data were assessed, and they were briefed on all study procedures. Afterwards, subjects were instrumented for continuous



recording of electroencephalography (EEG), electrooculography (EOG), and electromyography (EMG) and were trained on two different cognitive test batteries, each of which took approximately 25 min to complete. The first test battery consisted of the Wisconsin Card Sort Test, Thurstone's Word Fluency Test, and the Benton's Verbal Fluency Test, while the second test battery consisted of several cognitive and reaction time tasks, including a 2–3 min Serial Addition/Subtraction task, from the Walter Reed Performance Assessment Battery. Subjects were then transported to the General Clinical Research Center (GCRC), Johns Hopkins Bayview Medical Center, Baltimore, Maryland, where they began the residential portion of the study and continued to practice the cognitive tests. Subjects retired for sleep at 22.00 h and were awakened at 05.45 h on Day 2, and the same sleep schedule was followed for Days 2 and 3. Throughout Days 2 and 3, subjects practiced the cognitive performance tests, including the 2–3 min Serial Addition/Subtraction test (12 sessions total prior to the baseline PET scan). They were pretrained on the Serial Addition/Subtraction task and the other performance tests prior to the experimental sleep deprivation phase to hold learning constant. Also during Days 2 and 3, subjects took modified Multiple Sleep Latency Tests (MSLTs) and other physiological tests (e.g. oculomotor and vital signs monitoring). On the afternoon of Day 3, subjects attended the Johns Hopkins Radiochemistry and PET Scanning Facility at the Johns Hopkins Medical Institutions (JHMI) where they underwent a simulation PET scan procedure. This procedure included insertion of an antecubal IV catheter (which remained in-place and patent for the next four days), individual plastic face mask fitting, rehearsal of radiotracer injection, practice of the Serial Addition/Subtraction task for 30 min and subjective scales, and simulated PET scanning.

#### *Experimental phase*

On the morning of Day 4, after a night of normal sleep, subjects donned thermal underwear tops and bottoms, which were worn beneath their clothing, to keep them warm in order to facilitate the arterialization of venous blood flow through their hands for later venous blood draws (thermal clothing was then doffed after PET scanning and donned again the morning of the next PET scan). They took one modified MSLT between 07.00 and 08.00 h and ate a light breakfast, timed to maintain a 3-h fast prior to their designated  $^{18}\text{F}$ FDG injection. Subjects were next transported to the JHMI Radiochemistry and PET Facility for their baseline  $^{18}\text{F}$ FDG-PET scans.

Each 30-min  $^{18}\text{F}$ FDG injection and uptake occurred in the same room and in an enclosed tent-like structure that was erected to shield personnel associated with blood drawing and monitoring from the subjects' view. Prior to the  $^{18}\text{F}$ FDG uptake, subjects had a butterfly IV catheter inserted in the volar side of the left hand for blood drawing pre, during, and post  $^{18}\text{F}$ FDG injection and uptake. Their left hands were warmed with a heating pad to enhance blood flow and arterialize the venous blood. Thereafter, subjects took the

Stanford Sleepiness Scale (SSS) and the Global Vigor and Affect (GVA) scales (the latter included mood scales). Headphones were worn to attenuate transient background noise while they performed 5 min of the Serial Addition/Subtraction task as a 'warm up' to the uptake. Immediately prior to each  $^{18}\text{F}$ FDG injection, subjects were instructed to maintain wakefulness and to perform the task as quickly and accurately as possible. During and post  $^{18}\text{F}$ FDG injection, performance on the cognitive task continued for the 30 min of the uptake period. Task performance compliance was ascertained by monitoring subjects via video camera and wakefulness by monitoring their EEG via computer-based polygraph. Upon concluding the  $^{18}\text{F}$ FDG uptake, subjects completed another set of GVA scales and other visual analogue scales relating to sleep deprivation experiences. Afterwards, they relieved their bladders (to reduce radiation exposure to this target organ) and were carefully positioned in the PET scanner with their heads immobilized by an individually molded plastic face mask. Scanning then commenced for 30 min. Subjects began the 85 h of sleep deprivation following the baseline PET scans. They were scanned the following three days at the same time as their baseline scan (either 09.30, 10.30, 11.30, or 12.30 h).

During the time when subjects were not at the PET facility, they performed two cognitive test batteries (previously described) at alternate hours during the 85-h sleep deprivation period. As part of one of these cognitive test batteries, subjects performed a total of 8 sessions of the short-duration Serial/Subtraction task after the baseline PET scan and prior to the 24-h PET scan. Subjects continued to perform the cognitive test batteries after the fourth PET scan to preclude potential end spurt effects during the last  $^{18}\text{F}$ FDG uptake. Throughout the entire study, subjects were closely monitored by staff members, who administered test procedures and assisted in keeping them awake. Caffeine and other stimulants were not available to subjects during the study.

#### *Recovery phase*

Subjects received approximately 12 h of recovery sleep at the end of the 85-h sleep deprivation phase (19.00 to 06.45 h). On the morning of the last day, subjects took a modified MSLT, performed a set of the two cognitive test batteries, and completed the other physiological tests. At 10.00 h they were tested for 30 min on the Serial Addition/Subtraction task to assess recovery sleep effects on this performance measure. Following this, the subjects' electrodes were removed, and they were allowed to shower. They were then clinically assessed and de-briefed prior to departure from the study.

#### **Measures**

##### *Polysomnography (PSG)*

Scalp and facial electrodes were applied to: C3, C4, F3, F4, P3, P4, O1, O2, T3, and T4 for EEG; outer canthus of each eye for EOG; and submental for EMG. These signals were recorded continuously on Oxford Medilog 9000-II ambulatory recor-

ders (Oxford Medical Instruments, Hawthorne, New York, USA). Oxford Mentor laptop computers provided on-line, real-time output of PSG signals for monitoring sleep latency tests and verifying wakefulness during the  $^{18}\text{F}$ FDG uptake periods. Sleep periods during the study were scored in 30-sec epochs according to standard PSG criteria (Rechtschaffen and Kales 1968). Microsleep during the  $^{18}\text{F}$ FDG uptake was scored as theta, or stage 1 sleep, in the absence of artifact, with a duration of 1 to 15 sec. EEG from C3 was used for scoring theta events, and left and right EOG and EMG were used for assessing the presence of artifacts.

#### Neuroimage acquisition

Measurement of CMRglu was implemented according to standard practice and procedure (Reivich *et al.* 1979). Subjects were infused with a slow bolus, intravenous injection of  $^{18}\text{F}$ FDG (5 mCi per injection) in a right forearm vein. During the infusion and 30-min  $^{18}\text{F}$ FDG uptake period, subjects performed the Serial Addition/Subtraction task (see below). PET scanning then commenced (45 min post  $^{18}\text{F}$ FDG injection) and continued for a duration of 30 min. A GE 4096+ PET scanner (General Electric Medical Systems, Milwaukee, Wisconsin, USA) with an axial and in-plane resolution of 6.5 mm at full-width-half-maximum (FWHM) and a 15-cm field of view was used to acquire the distribution of radioactivity in the brain. Emission data were corrected for attenuation using a transmission scan obtained at the same levels. Attenuation-corrected data were reconstructed into 15 image planes. As indicated above, a heating pad was used to warm the subject's left hand to 44 °C to transform the pH,  $\text{P}_{\text{O}_2}$ ,  $\text{P}_{\text{CO}_2}$ , and glucose levels in the venous blood to values more nearly resembling those of arterial blood. Samples of arterialized-venous blood were drawn at fixed intervals throughout each uptake and imaging procedure and were used to transform radioactivity counts to CMRglu (Phelps *et al.* 1979). Repositioning of the subjects on the PET scanner between the experimental days was accurate to within 2 mm.

#### Alertness test

Objective alertness was assessed using a modified version of the MSLT (Carskadon *et al.* 1986). Subjects were allowed to sleep in a quiet, darkened bedroom until they reached stage 2 sleep or after 20 min had elapsed. Sleep latency was defined as the elapsed time to the first 30 sec of stage 2 sleep.

#### Self-reports

Subjects' self-ratings of sleepiness were assessed with a computerized version of the Stanford Sleepiness Scale (SSS) (Hoddes *et al.* 1973). The SSS is a one-item choice scale consisting of seven numbered statements that describe alertness states ranging from 1 ('feeling active and vital; alert; wide awake') to 7 ('almost in reverie; sleep onset soon; losing struggle to remain awake'). Self-rated levels of effort and motivation in Serial Addition/Subtraction performance were

assessed using visual analogue scales (single straight 10 cm horizontal lines scored between 0 and 100). Visual analogue scales relating to vigor and affect (Monk 1989), and sleep deprivation experiences (data not reported) were also acquired near the  $^{18}\text{F}$ FDG uptake periods.

#### Cognitive task

The Serial Addition/Subtraction task (Thorne *et al.* 1985) consists of two randomly selected single digits (0–9) and an operator (either + or – sign) displayed sequentially in the same center-screen location, followed by a '?' prompt. The subject performs the indicated addition or subtraction and, if the result is positive, enters the least significant digit of the result. If the result is negative, the subject adds 10 and enters the positive single digit result. The digits and operator are each presented for 250 msec, with a 200 msec interdigit/operator interval. The next trial begins 300 msec after a key entry, or response, is made by the subject. Consequently, there is no opportunity for an omission, or lack of response. The 200 possible combinations of two digits with two operators were randomly sampled several times during each  $^{18}\text{F}$ FDG uptake, and hence, were essentially of equal difficulty. Consistent for each uptake period, the task was divided into six, 5-min segments, to document time-on-task effects as well as to provide periodic feedback of performance results to the subjects (visually on the computer monitor).

#### Data analysis

##### PET data

Statistical parametric mapping (SPM) software (SPM95, Wellcome Department of Cognitive Neurology, London, UK) was used for registering and statistically analysing the PET data (Friston *et al.* 1995, 1996). The 15 original axial PET planes were trilinearly interpolated to yield 43 planes in which voxels (3-D picture elements [pixels] in neuroanatomical space) were approximately cubic. To minimize the effects of head displacement, the scans of each subject were realigned to the first PET scan on a voxel-by-voxel basis using the SPM routine employing a rigid body spatial transformation. Next, the PET scans of all of the subjects were transformed into standard stereotaxic space using both linear and nonlinear three-dimensional transformation methods to allow for voxel-by-voxel averaging across subjects. The stereotaxically normalized scans consisted of 26 planes (voxel size  $2 \times 2 \times 4$  mm) corresponding to the brain atlas of Talairach and Tournoux (1988). Images were smoothed using a 12 mm Gaussian filter to accommodate intersubject differences in gyral and functional anatomy and to increase the signal-to-noise in the images. This produced a final image resolution of  $19 \times 20 \times 17$  mm.

To evaluate quantifiable changes in regional CMRglu that occurred during the progression of sleep deprivation, the absolute rates of regional CMRglu during sleep deprivation and the rested baseline were analysed and compared. The global normalization parameter was not used in the absolute regional CMRglu analysis. Global CMRglu values (i.e. means)

were obtained for each subject's PET scans from the absolute regional CMRglu analysis. The difference between days was analysed using a one-tailed paired *t*-test, with a Bonferonni adjustment applied based on the number of comparisons across the entire experimental sleep deprivation phase. Absolute regional CMRglu effects were obtained from the transformation of one-tailed *t*-tests to the *Z* probability distribution. Also, relative regional CMRglu effects were analysed in the same way after covarying out the effect of global CMRglu using ANCOVA and normalization of the values relative to 5.4 mg/100 g · min. The resulting *Z*-values comprised a statistical parametric map SPM(*Z*). For both absolute and relative regional CMRglu comparisons, the SPM was thresholded for statistical significance at  $P \leq 0.001$ , uncorrected for multiple comparisons ( $Z \geq 3.09$ ), for regions predicted to change a priori (thalamus and prefrontal cortex) or which had been shown to significantly change in the Wu *et al.* (1991) sleep deprivation study of CMRglu (temporal cortex, thalamus, and cerebellum). A threshold of  $P \leq 0.05$  corrected for multiple comparisons ( $Z \geq 4.16$ ) was used for nonhypothesized regions. Individually acquired MRI scans showed that each subject's neuroanatomy was normal (i.e. without signs of disease or atrophy). The high resolution MRI scan of a normal male brain provided in the SPM program was subsequently used to identify neuroanatomical locations of functional change for the group.

#### Behavioral data

Sleep latency, self-report, and cognitive performance data were analysed using one-tailed paired *t*-tests, with the exception that subjectively rated mood data were analysed using two-tailed paired *t*-tests. Bonferonni adjustments were applied to the behavioral data. Self-report data were log transformed prior to statistical analysis. Correlation analyses between behavioral measures and regional CMRglu are planned for a future report.

## RESULTS

### Polysomnography

#### Scheduled sleep

Subjects obtained an average of 396 min (6 h and 36 min) of sleep the night before the baseline PET scan. This amount is equivalent to that obtained for each adaptation night. Sleep stage distribution was consistent for all nights prior to the sleep deprivation period. The sleep parameters for all nights were within the range for normal sleep (sleep onset  $\leq 30$  min, sleep efficiency  $\geq 90\%$ , and number of arousals  $\leq 30$ ).

#### Unscheduled sleep on PET scanner

Subjects obtained an average of 14 min of unscheduled sleep during the baseline rested PET scan. The sleep occurred when subjects were required to remain motionless on the scanner for 30 min to ensure successful image acquisition. The regional CMRglu activity imaged by the scanner reflects brain activity

during the  $^{18}\text{F}$ FDG uptake and not brain activity during image acquisition. The amount of sleep acquired on the scanner represents 1% of the total 24 h sleep deprivation period. The resultant sleep consisted primarily of stage 1 and occurred approximately 24 h prior to the 24-h sleep deprivation  $^{18}\text{F}$ FDG uptake.

#### Wakefulness during $^{18}\text{F}$ FDG uptakes

Post hoc analysis of the recorded polysomnographic signals showed that all subjects were awake during the 30-min  $^{18}\text{F}$ FDG uptake period by polysomnographic criteria. The amount of microsleep was negligible and occurred during both the rested (mean = 2 sec) and 24-h sleep deprivation (mean = 5 sec)  $^{18}\text{F}$ FDG measurements.

### Brain Activity

#### Global CMRglu

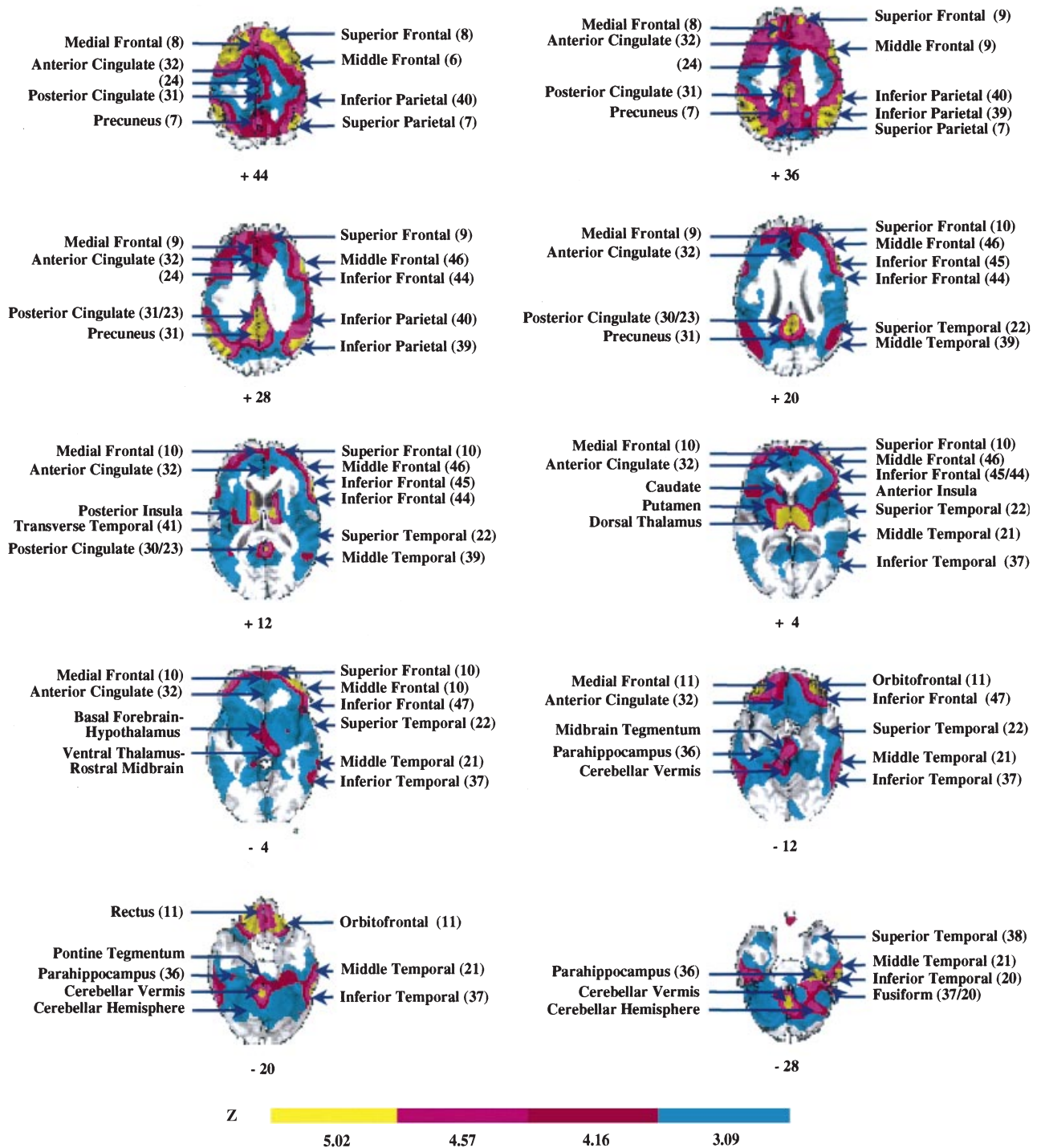
Global CMRglu, expressed as the average of all voxels (excluding white matter), decreased by approximately 8% (actual 7.76%) after 24 h of sleep deprivation [5.67 milligrams/100 g · min (31.4  $\mu\text{mol}/100\text{ g} \cdot \text{min}$ ), rested PET scans vs. 5.23 milligrams/100 g · min (29.0  $\mu\text{mol}/100\text{ g} \cdot \text{min}$ ), 24-h sleep deprived PET scans;  $t = 3.78$ ,  $P \leq 0.001$ ].

#### Decreases in regional CMRglu

Following 24 h of sleep deprivation, significant decreases in absolute regional CMRglu were observed for numerous brain regions (Fig. 2). As revealed by significant decreases in relative regional CMRglu (Fig. 3), there was heterogeneity in regional brain activity response during sleep deprivation. Table 1 shows that at the same voxel location for relative regional CMRglu, the decreases in absolute regional CMRglu were approximately 3–7% greater than the 8% decrease in global CMRglu. This indicates that regions that significantly decreased in relative regional CMRglu were more affected than those which decreased at the global or whole brain level. Hemispheric analyses revealed no statistically significant laterality differences in either absolute or relative regional CMRglu with 24 h of sleep deprivation.

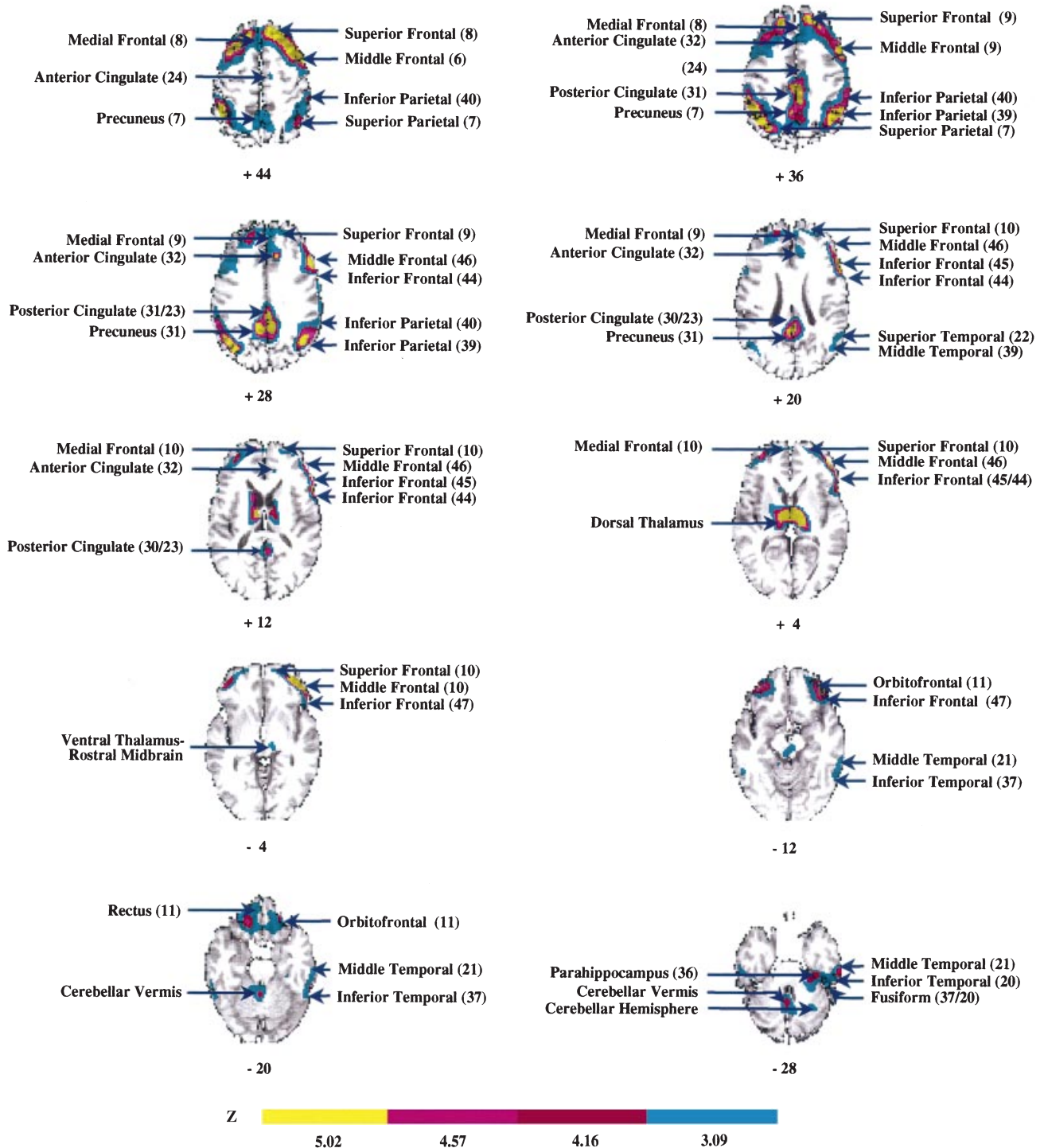
For hypothesized regions, decreases in absolute regional CMRglu occurred bilaterally throughout the prefrontal cortex (including dorsal and ventral anterior cingulate gyri), and in the dorsal and ventral thalami after 24 h of sleep deprivation. Additionally, absolute decreases occurred bilaterally in the temporal lobes and parahippocampal gyri, as well as the cerebellar hemispheres and vermis. Decreases in relative regional CMRglu occurred bilaterally in the prefrontal cortex (including dorsal anterior cingulate gyrus) and in the thalamus. Also, decreased regional CMRglu was observed in the middle and inferior temporal gyri, in medial temporal cortex consisting of the right fusiform and parahippocampal gyri, in the cerebellar vermis, and in a small area in the right ventral cerebellar hemisphere.





**Figure 2.** Significant decreases from baseline in absolute regional CMRglu during wakefulness and cognitive task performance after 24 h of sleep deprivation across 17 subjects. Deactivations are superimposed on a single subject's magnetic resonance imaging (MRI) template. Axial images are oriented in millimeters relative to the anterior commissure-posterior commissure (AC-PC) plane. The left/right hemispheres appear as the left/right sides of each image. Significant regions are color coded to reflect thresholds for statistical probability levels: 5.02 = 0.001 corrected, 4.57 = 0.01 corrected, 4.16 = 0.05 corrected, 3.09 = 0.001 uncorrected. Thresholds for statistical significance are  $Z \geq 3.09$  for regions predicted to decrease *a priori* (thalamus and prefrontal cortex) and/or previously published for short-term sleep deprivation effects on regional CMRglu (temporal cortex, thalamus, and cerebellum [Wu *et al.* 1991]), and  $Z \geq 4.16$  for nonhypothesized regions. Statistically significant regions are neuroanatomically labeled and approximate Brodmann areas (BAs) are noted in parenthesis ( ).





**Figure 3.** Significant decreases from baseline in relative regional CMRglu during wakefulness and cognitive task performance after 24 h of sleep deprivation across 17 subjects. Details are the same as for Fig. 2. Decreases in relative regional CMRglu resulting from sleepiness, while spatially smaller, actually represent the areas with the largest reductions in absolute regional CMRglu (i.e. 3–7% greater decreases in absolute regional CMRglu than the global CMRglu decrease of approximately 8%; see Table 1 for direct comparison of voxel locations and percent decreases between relative and absolute regional CMRglu).

Several nonhypothesized regions also evidenced decreases in absolute regional CMRglu, such as the lateral posterior parietal (both inferior and superior lobules) and the medial parietal cortices (including posterior cingulate gyrus and precuneus), the right anterior and left posterior insula,

caudate, putamen, globus pallidus, basal forebrain-hypothalamus, midbrain tegmentum, and mesopontine and pontine tegmentum. Significant decreases in relative regional CMRglu were also apparent throughout the posterior parietal lobes.

**Table 1** Significant decreases from baseline in regional CMRglu during wakefulness and cognitive task performance after 24 h of sleep deprivation (*n* = 17\*)

Region†	Left Hemisphere						Right Hemisphere						
	BA‡	<i>x, y, z coordinates</i> §	Relative Regional CMRglu		Absolute Regional CMRglu		<i>x, y, z coordinates</i>	Relative Regional CMRglu		Absolute Regional CMRglu			
			Z¶	% Δ	Z	% Δ		Z	% Δ	Z	% Δ		
Frontal Cortex – Lateral													
Middle Frontal Gyrus	6	-38, 10, 44	4.99	5.65	5.53	12.83	36, 10, 44	6.25	5.38	5.57	12.94		
Superior Frontal Gyrus	8	-16, 32, 44	6.03	5.05	5.47	12.61							
Middle Frontal Gyrus	8	-16, 40, 36	5.72	3.85	5.14	11.31	34, 16, 44	7.26	5.02	5.46	13.03		
Superior Frontal Gyrus	9						32, 14, 40	5.52	4.05	5.12	12.00		
Middle Frontal Gyrus	44	-50, 8, 28	4.18	2.90	4.74	10.69	46, 16, 24	5.90	3.73	5.08	11.47		
Inferior Frontal Gyrus	45	-46, 34, 0	2.99††	2.14	4.44	9.89	50, 16, 20	5.57	4.78	5.35	12.44		
Middle Frontal Gyrus	46	-34, 48, 4	4.96	3.18	4.88	10.86	40, 42, 0	6.58	5.96	5.78	13.34		
Middle Frontal Gyrus	10	-34, 48, 8	4.89	3.07	4.85	10.75	34, 46, -4	6.84	6.92	6.07	14.14		
Middle Frontal Gyrus	47	-40, 38, -8	5.15	4.71	5.37	11.82	38, 44, -4	7.14	7.22	6.13	14.61		
Orbitofrontal Gyrus	11	-34, 38, -12	5.16	6.36	5.74	13.22	36, 40, -12	5.50	7.30	5.76	15.52		
Frontal Cortex – Medial													
Medial Frontal Gyrus	8	-12, 38, 40	4.76	3.11	4.84	10.88	10, 38, 40	6.19	4.47	5.29	12.30		
	9	-16, 42, 32	4.87	3.14	4.88	10.71	14, 44, 32	4.54	3.11	4.78	11.33		
	10	-14, 58, 12	4.53	3.88	4.99	11.91	16, 56, 0	4.31	3.22	4.81	11.28		
Rectus Gyrus	11	-12, 24, -20	4.36	6.26	5.44	13.72	12, 16, -20	4.04	5.01	5.12	13.11		
Parietal Cortex – Lateral													
Superior Parietal Lobule	7	-36, -74, 32	6.02	5.08	5.50	12.52	38, -62, 40	5.49	5.72	5.56	13.33		
Inferior Parietal Lobule													
Supramarginal Gyrus	40	-50, -50, 40	6.11	6.88	5.87	14.57	44, -58, 32	5.55	4.72	5.26	12.86		
Angular Gyrus	39	-42, -68, 32	6.14	5.07	5.46	12.78	42, -58, 36	6.05	5.74	5.61	13.51		
Parietal Cortex – Medial													
Precuneus	7	-10, -56, 32	5.97	4.17	5.19	12.04	2, -44, 32	5.73	4.35	5.28	11.81		
	31	-4, -54, 24	5.41	4.61	5.35	11.85	6, -54, 24	5.44	4.23	5.24	11.64		
Temporal Cortex – Lateral													
Transverse Temporal Gyrus	42	-36, -26, 12	§§	§§	4.05	8.78	36, -26, 12	§§	§§	4.20	9.43		
Superior Temporal Gyrus	22	-58, -48, 20	2.76††	2.32	4.45	10.21	56, -50, 20	2.94††	2.29	4.43	10.81		
	38	-22, 4, -24	§§	§§	3.95	11.43	22, 4, -24	§§	§§	3.41	10.54		
Middle Temporal Gyrus	39	-44, -70, 24	5.53	3.56	5.00	11.40	40, -70, 24	4.89	3.16	4.89	10.71		
	21	-58, -44, -16	3.74	4.76	4.95	13.62	54, -48, -16	4.29	5.32	5.29	12.78		
Inferior Temporal Gyrus	37	-58, -46, -16	3.79	4.97	5.00	13.69	52, -46, -20	4.44	5.53	5.32	13.29		
	20	-56, -24, -28	4.06	3.81	4.94	11.56	54, -26, -28	4.65	6.02	5.39	14.26		
Temporal Cortex – Medial													
Fusiform Gyrus	37	-52, -44, -24	2.62††	3.53	4.52	12.53	48, -44, -24	3.76	5.36	5.10	13.37		
	20	-42, -32, -28	3.28	5.26	4.92	13.41	42, -40, -28	3.79	5.21	5.07	13.51		
Parahippocampal Gyrus	36	-34, -32, -4	2.92††	3.51	4.62	11.93	26, -30, -28	4.61	6.46	5.59	13.41		



<i>Test</i>	<i>Rested Baseline</i>	<i>24 h of Sleep Deprivation</i>	<i>t</i>	<i>P</i>
Modified Multiple Sleep Latency Test				
Elapsed time to stage 2 (min:sec)	18:12 (04:46)	03:26 (01:39)	11.41	0.000
Stanford Sleepiness Scale (1–7)	1.8 (1.0)	2.9 (1.2)	– 3.04	0.004
Global Vigor and Affect Scales (0–100)				
Pre- <sup>18</sup> FDG Uptake				
Vigor – Alert	88.7 (17.8)	62.9 (24.7)	4.27	0.000
Vigor – Effort	11.5 (13.8)	33.5 (24.3)	– 4.22	0.000
Vigor – Weary	13.3 (18.0)	40.1 (25.2)	– 4.54	0.000
Vigor – Sleepy	10.9 (15.9)	46.1 (26.6)	– 5.50	0.000
Affect – Sad	12.8 (24.8)	5.7 (10.7)	0.83	0.42
Affect – Tense	38.4 (36.8)	41.3 (36.4)	– 0.19	0.85
Affect – Happy	63.5 (27.3)	62.1 (19.6)	0.20	0.85
Affect – Calm	60.7 (28.1)	60.8 (31.0)	0.45	0.66
Post- <sup>18</sup> FDG Uptake				
Vigor – Alert	71.8 (21.9)	44.1 (23.3)	3.71	0.001
Vigor – Effort	23.4 (24.9)	53.7 (29.3)	– 4.86	0.000
Vigor – Weary	22.6 (27.0)	50.6 (35.6)	– 3.30	0.002
Vigor – Sleepy	22.0 (23.4)	68.1 (23.3)	– 6.03	0.000
Affect – Sad	2.2 (4.6)	5.2 (8.6)	– 1.66	0.12
Affect – Tense	23.5 (34.0)	27.3 (32.1)	– 1.65	0.12
Affect – Happy	64.0 (28.9)	54.8 (28.6)	0.95	0.36
Affect – Calm	60.5 (33.4)	64.7 (27.1)	0.70	0.50
Post- <sup>18</sup> FDG/Cognitive Performance Scales (0–100)				
Effort	54.7 (32.0)	74.7 (26.6)	– 3.06	0.004
Motivation	78.7 (30.5)	82.0 (28.4)	– 1.04	0.16
Serial Addition/Subtraction Task during <sup>18</sup> FDG Uptake (30 min total)				
Accuracy (% correct)	95.5 (5.2)	92.3 (6.4)	2.97	0.005
Speed (responses/min)	71.0 (27.2)	61.4 (24.6)	3.48	0.002
Throughput (correct responses/min)	68.3 (27.3)	57.5 (25.2)	3.54	0.001

Values are mean ± standard deviation.

### *Increases in regional CMRglu*

No significant increases in absolute regional CMRglu, nor trends for significant increases, were noted with 24 h of sleep deprivation. Therefore, increases in relative regional CMRglu (data not shown), which were evident after covarying out the global CMRglu decrease, reflected either a lack of statistically significant decrease in absolute regional CMRglu or invariance in regional CMRglu: left postcentral gyrus (BAs 3, 4); left/right lateral occipital cortices (BAs 18, 19); left superior temporal cortex (BA 22); left/right lingual and fusiform gyri (BAs 18, 19); right mesial temporal lobe (amygdala area, BA 28); and right dorsal cerebellar lobe.

### **Behavior**

After 24 h of sleep deprivation, objective and subjective alertness declined but mood remained constant (Table 2): latency to stage 2 sleep significantly decreased on the modified MSLT, sleepiness ratings on the SSS significantly increased, and significant changes were found for all vigor-related scales of the GVA instrument indicating increased sleepiness and

**Table 2** Alertness, self-assessments, and cognitive performance during wakefulness after a night of normal sleep (rested baseline) and 24 h of sleep deprivation ( $n = 17$ )

effort to remain awake and perform. Significant changes, however, were not found for any of the mood-related scales of the GVA instrument after sleep deprivation. Analysis of other visual analogue scales revealed that after sleep deprivation, subject-perceived effort to perform the Serial Addition/Subtraction task during the <sup>18</sup>FDG uptake increased significantly, while subjective ratings of motivation to perform the task remained consistently high (Table 2).

A significant reduction was observed after sleep deprivation in cognitive performance on the Serial Addition/Subtraction task during the <sup>18</sup>FDG uptake with respect to accuracy, speed, and throughput (Table 2): accuracy decreased by 3%, speed by 13%, and throughput, a speed-accuracy product and index of overall productivity (Thorne *et al.* 1983), by 16%. Table 3 shows that within the 30-min Serial Addition/Subtraction task during the rested baseline <sup>18</sup>FDG uptake, there was no significant time-on-task decrease in performance when each subsequent 5 min segment was compared with the first segment. In the 24-h sleep deprivation session, each subsequent segment was significantly different than the first segment for all three performance measures. This time-on-task effect was linear over the first 15 min and then remained stable for the remaining 15 min.



**Table 3** Time-on-task 30 min performance during  $^{18}\text{F}$ FDG uptake periods (5 min segments) after a night of normal sleep (rested baseline) and 24 h of sleep deprivation ( $n = 17$ )\*

Serial Addition/ Subtraction Task	Rested Baseline		24 h of Sleep Deprivation			
		<i>t</i>	<i>P</i>		<i>t</i>	<i>P</i>
Accuracy (% correct)						
1st 5 min	95.5 (4.7)			96.1 (3.0)		
2nd 5 min	96.5 (3.4)	- 1.50	0.08	93.9 (5.4)	2.41	0.01
3rd 5 min	95.4 (4.2)	0.13	0.45	92.2 (7.4)	2.98	0.004
4th 5 min	94.3 (9.7)	0.67	0.26	90.9 (8.7)	3.31	0.002
5th 5 min	95.6 (7.4)	- 0.08	0.47	90.5 (8.7)	3.30	0.002
6th 5 min	95.9 (5.6)	- 0.31	0.38	90.1 (8.9)	3.27	0.002
Speed (response/min)						
1st 5 min	72.2 (28.4)			71.6 (29.2)		
2nd 5 min	69.3 (25.3)	1.29	0.11	63.3 (27.9)	2.95	0.005
3rd 5 min	72.4 (32.3)	- 0.07	0.47	59.5 (28.5)	3.10	0.003
4th 5 min	68.9 (28.2)	1.45	0.08	57.9 (26.4)	3.30	0.002
5th 5 min	70.7 (25.2)	0.58	0.28	57.6 (20.3)	3.05	0.004
6th 5 min	72.7 (28.4)	- 0.25	0.41	58.7 (23.9)	3.41	0.002
Throughput (correct responses/min)						
1st 5 min	69.3 (28.5)			69.3 (29.3)		
2nd 5 min	67.2 (25.9)	0.94	0.18	60.1 (28.0)	3.20	0.003
3rd 5 min	69.7 (32.3)	- 0.14	0.46	55.7 (29.0)	3.42	0.002
4th 5 min	65.7 (28.7)	1.69	0.05	53.6 (27.4)	3.69	0.001
5th 5 min	67.9 (25.1)	0.54	0.30	52.5 (20.7)	3.83	0.001
6th 5 min	70.0 (28.4)	- 0.35	0.37	53.8 (25.2)	3.99	0.001

\* Statistical comparisons are between the first 5-min segment and each of the last five 5-min segments within each day.

Values are mean  $\pm$  standard deviation.

## DISCUSSION

### Implications for sleep deprivation-induced alertness and cognitive performance decrements

Concurrent with impaired alertness and cognitive performance, 24 h of sleep deprivation produced a decrease in global CMRglu during polysomnographically defined wakefulness, and decreased absolute regional CMRglu in several cortical and subcortical regions. Increases in absolute regional CMRglu were not observed in any region. Decreases in relative regional CMRglu – indicating areas more deactivated than the global decrease – were found throughout the thalamus and prefrontal cortex. These brain regions subserve alertness and attention, while the prefrontal cortex also mediates the highest-order cognitive processes, the mental abilities most impaired by sleep deprivation. Extensive decreases in absolute and relative regional CMRglu were found throughout another cortical region, the posterior parietal lobes, following sleep deprivation. The vast deactivations in the prefrontal and posterior parietal cortices included the heteromodal association areas (BAs 8, 32, 45, 46, 9, 10, 11 in the prefrontal cortex and BAs 7, 40, 39 in the posterior parietal cortex), which are involved in the higher-order analysis and integration of sensory-motor information and cognition (Mesulam 1985).

The finding of thalamic deactivation after 24 h of sleep deprivation in the present study is highly consistent with this structure's role in cerebral activation and alertness (Roland 1993) and with the measured decrease in sleep latency and increase in subjective sleepiness. Decreased regional CMRglu

in the thalamus has also been observed following extreme sleepiness in rats (Everson *et al.* 1994) and has been found to negatively correlate with benzodiazepine-induced sleepiness during wakefulness (Volkow *et al.* 1995). Moreover, the thalamus is important to task performance requiring attention and alertness (Kinomura *et al.* 1996; Shulman *et al.* 1997), and reduced activity of this region following sleep deprivation may have contributed to deficits in attention during Serial Addition/Subtraction performance. Other evidence supporting this view is that thalamic deactivation has been found in previous studies of sleep deprivation and impaired attentional performance (Wu *et al.* 1991; Drummond *et al.* 1999a) and has been shown to coincide with attention and vigilance deficits in patients with fatal familial insomnia, a disease characterized by thalamic lesions and intractable insomnia (Perani *et al.* 1993).

Anatomically, the thalamus has known bi-directional connections with the prefrontal-posterior parietal cortical association areas, which were also substantially deactivated by sleep deprivation. The thalamus and these cortical regions are considered to be part of a distributed neural network for directed attention (Mesulam 1990), and studies have demonstrated coactivation of these three areas during tasks requiring sustained attention (Coull *et al.* 1996, 1998) and intrinsic alertness (Sturm *et al.* 1999). Of particular relevance is that decreased activity in these structures has been found for degraded time-on-task vigilance performance during normal alertness (Paus *et al.* 1997). Time-on-task impairments in performance are well-documented in sleep deprivation studies (Johnson 1982) and were found with sleep deprivation in the current study.

In addition to contributing to attentional processes, cerebral activation studies show that under normal alertness conditions the prefrontal and posterior parietal cortices are recruited during tasks requiring visual verbal working memory (Coull *et al.* 1996; Dolan *et al.* 1997) and arithmetic calculations (Roland and Friberg 1985; Dohaene *et al.* 1996, 1999). Behaviorally, these cognitive functions are also necessary for Serial Addition/Subtraction performance, which decreased in conjunction with deactivation of these cortical areas after 24 h of sleep deprivation. Due to the long half life (110 min) of  $^{18}\text{F}$ FDG, a control task was not evaluated to directly discern the functional brain components of the Serial Addition/Subtraction task. A control task was evaluated in the Drummond *et al.* (1999a) sleep deprivation study using a similar arithmetic task, which revealed task-related relative activations in localized areas of the prefrontal and posterior parietal regions during rested arithmetic performance. The deactivations in the prefrontal cortex in the present study were more extensive than those observed in that sleep deprivation study, however, and therefore may have functional implications beyond simply reflecting the cognitive task used. The prefrontal cortex mediates other higher-order mental abilities impaired by sleep deprivation (see Introduction), such as verbal fluency, speech, flexible and innovative thinking, and planning, judgment, and decision making based on new or updated information (Fuster 1989; Roland 1993; Damasio 1994; Frith and Dolan 1996). The magnitude and amount of reduced activity found in this region suggest that other higher-order cognitive impairments proposed by Horne (1988b, 1993) and noted in various sleep deprivation experiments could be a consequence of, and explained by, declines in prefrontal cortical functioning.

#### **Reconciliation with other brain activity findings of human sleep deprivation**

Our brain activity results for sleep deprivation confirm several findings from a previous study of absolute and relative regional CMRglu changes of short-term sleep deprivation and visual attention deficits (Wu *et al.* 1991). We found a decrease in global CMRglu of approximately 8% compared with a 7% decrease observed by Wu *et al.* (1991), albeit theirs did not reach statistical significance. We also found deactivation of the temporal lobes, thalamus, and cerebellum. Likewise, we noted increases in relative regional CMRglu in occipital cortex, which reflected nonsignificant decreases in absolute regional CMRglu. In contrast, we found significant decreases in absolute and relative regional CMRglu throughout the prefrontal cortices, including anterior cingulate gyrus, and the posterior parietal cortices, including posterior cingulate gyrus and precuneus. Decreases in absolute regional CMRglu in basal ganglia, basal forebrain, and midbrain and pontine tegmentum brainstem areas were also apparent. Incongruity in findings between the two investigations may be explained in part by differences in the image analysis procedures used as already described. Evidence of this is given by a recent report (Wu *et al.* 1999), where the authors applied a more sensitive

analysis to their data and showed decreases in regional CMRglu in right dorsolateral prefrontal cortex (BA 46).

Apart from the analysis procedure, the differences in regional brain activity results between the studies might be explained by apparent differences in task demands (i.e. level of difficulty related to rate of stimulus presentation and cognitive processing) and complexity (i.e. type of mental processing, such as memory and arithmetic processing) necessitated by the two different tasks used to probe brain function during sleep deprivation. In the present study, Serial Addition/Subtraction performance involved sustained attention, working memory and arithmetic calculations of all fast-paced stimulus presentations, whereas in the Wu *et al.* (1991) study, Continuous Performance Test performance required sustained attention and a vigilance component, necessitating identification responses when an infrequently occurring target stimulus appeared (Mesulam 1985). While both sleep deprivation studies produced performance impairments, the rate and nature of task requirements imposed by the Serial Addition/Subtraction task is arguably more difficult and complex than those imposed by the Continuous Performance Test. Subjects in our study reported not only a moderate amount of effort to perform this task when well-rested but also significant increases in effort over baseline with sleep deprivation, substantiating that the task became more difficult to perform when sleepy.

The idea that brain activity response to sleep deprivation could be task and/or task outcome specific has been noted previously (Thomas 1997; Drummond *et al.* 2000). In fact, our findings of decreased activity in prefrontal anterior cingulate cortex, lateral posterior parietal cortices, and thalamus during sleep deprivation and decreased Serial Addition/Subtraction task performance are in agreement with findings on a similar, but of shorter-duration, complex cognitive task resulting in performance impairment (Drummond *et al.* 1999a). Support for task-specific neural responses, with and without performance impairment, has been demonstrated in other short-term sleep deprivation studies (Portas *et al.* 1998; Smith *et al.* 1999; Drummond *et al.* 1999b; 2000). The results of studies where performance was held constant revealed either no change or primarily increases in regional brain activity. However, the tasks used may not have been of sufficient cognitive load or challenge to evoke diminished neural responses. This concept was evaluated in a study where differences in dorsolateral prefrontal cortical activation between schizophrenic patients and controls became apparent only after difficulty on a memory task increased and performance impairment occurred (Fletcher *et al.* 1998). Nonetheless, in addition to task difficulty and task complexity characteristics, task duration characteristics may play an important role in delineating brain activity responses during sleep deprivation. Differences in time-on-task performance are likely to occur between studies using different designs and scanning methods (and hence different task durations) to assess sleep deprivation brain activity effects; for example, temporal differences between a 30-min  $^{18}\text{F}$ FDG uptake acquisition, in which the first 10 min accounts for a

majority of the regional brain activity response, vs. multiple, 40-sec scans for a BOLD-fMRI response acquisition.

Based on the results of the above studies of sleep deprivation, these brain imaging data suggest that following one night of sleep deprivation, neurons have the capacity to respond normally when the brain is presented with a nonchallenging task, or may be able to temporarily increase their response in specific regions in an attempt to meet the demands of simple short-term task performance. In the case of complex task performance and/or sustained task performance during sleep deprivation, the findings of decreased regional brain activity suggest that neurons cannot keep pace with high task load requirements and/or neuronal responsiveness is diminished or fatigued after a certain period of performance (i.e. a time-on-task effect) thereby resulting in a decrement in task outcome.

### Comparisons with regional brain activity alterations observed during human sleep

An intriguing implication of the results from the current functional neuroimaging study of sleep deprivation, when compared with results for sleep, is that the larger decreases in activity in the prefrontal and posterior parietal heteromodal association cortices may indicate a greater biological vulnerability of these areas to extended wakefulness. Other work from our laboratory (Balkin *et al.* 1992; Braun *et al.* 1997) has shown absolute and relative decreases in dorsolateral prefrontal and inferior parietal cortical activity (as measured by cerebral blood flow) during light sleep, slow wave sleep and REM sleep, which has also been reported by others (e.g. Buchsbaum *et al.* 1989; Andersson *et al.* 1998; Maquet *et al.* 1996; Kajimura *et al.* 1999). Thus, the same higher-order cognitive areas differentially affected by sleep deprivation are also differentially affected by sleep indicating that these areas may be more susceptible to sleep deprivation and consequently have a greater need for the recuperative processes underlying sleep. Such homeostatic processes may include brain energy substrate and neuromodulator replenishment (Benington and Heller 1995; Newhouse *et al.* 1989; McCann *et al.* 1992, 1993, 1995) and/or adjustment of ionic currents and reorganization of network patterns of synaptic activity as a consequence of learning (Steriade *et al.* 1993).

Neuroimaging studies of sleep have also uniformly revealed decreased activity in the thalamus when measured during light and/or deep NREM sleep (e.g. Buchsbaum *et al.* 1989; Balkin *et al.* 1992; Maquet *et al.* 1990, 1992, 1997; Braun *et al.* 1997; Hofle *et al.* 1997; Andersson *et al.* 1998; Kajimura *et al.* 1999). Complementary to this, we showed that the largest subcortical deactivation in waking regional CMRglu after one night of sleep deprivation occurred in the thalamus, and this has been a relatively consistent result in other neuroimaging studies of sleep deprivation (Wu *et al.* 1991; Everson *et al.* 1994; Drummond *et al.* 1999a). Taken together, these findings suggest that a progressively deactivated thalamus may be necessary for the transition from waking to sleep and for the occurrence of deeper stages of sleep.

### Temporal occurrence of sleep deprivation-induced deactivations

Given the limited temporal resolution of PET-based brain imaging, we cannot determine the source of our sleep deprivation-induced brain deactivation. Deactivation could have originated in the thalamus, in the cortex, or in other more caudal brain regions known to be involved in thalamic and cortical activation (McCormick and Bal 1997). Thus far, decreased corticothalamic activity is the most marked brain alteration seen with human sleep deprivation, whereas activity in the areas of the basal forebrain and mesencephalon and pontine tegmentums is either less affected as in the current study or observed not to change significantly (Wu *et al.* 1991; Drummond *et al.* 1999a,b; 2000). These latter areas, specifically the small nuclei associated with promoting thalamic and cortical activation and those associated with promoting sleep in animals (Steriade and McCarley 1990; Szymusiak 1995), will require higher spatial, as well as temporal, resolution scanners to accurately identify their involvement in human sleepiness.

Further data analyses of the present study, to include correlation analysis of alertness and cognitive performance alterations with the changes in regional brain activity, as well as the neuroimaging measures associated with the 48 and 72 h sleep deprivation time points, may shed light on regions which may be affected directly or might be most sensitive to sleep deprivation.

### CONCLUSIONS

One night of sleep deprivation in humans diminishes waking regional brain activity predominantly in a bilateral prefrontal-posterior parietal-thalamic network mediating alertness attention and higher-order cognitive processes. The cortical association findings are complementary to studies of slow wave and REM sleep demonstrating deactivation of these same cortical regions, with the implication that the need for recuperation during sleep may be greater in these areas relative to other brain regions. Our results of brain activity, alertness, and cognitive performance impairments following one night of sleep deprivation suggests that the neurobehavioral function of sleep in humans is to restore and sustain normal waking brain activity and behavior. These findings substantiate the biological necessity of sleep to normal brain functioning and are particularly powerful in underscoring the importance of adequate sleep for workplace productivity, public safety, and personal well being.

### ACKNOWLEDGEMENTS

Funding was provided by the Military Operational Medicine Program, Project #S15 Q, U.S. Army Medical Research and Materiel Command, Ft. Detrick, Maryland, and by the GCRC/Johns Hopkins Bayview Medical Center, Grant #M01RR02719, Baltimore, Maryland. Technical support was also provided by Science Applications International, Inc. (SAIC) through Contract #MDA903-92-0068 with the

Human Research and Engineering Directorate, US Army Research Laboratory, Aberdeen Proving Ground, Maryland.

We thank our anonymous reviewers for their thoughtful comments and suggestions. We also thank our volunteers for their participation. For technical assistance, we thank the following: Johns Hopkins Radiochemistry and PET staff, Walter Reed enlisted military and student contract staff, GCRC/Johns Hopkins Bayview research nursing staff (J. Wright, Nursing Supervisor; P. Knighton, Study Manager), and staff at Henry M. Jackson Foundation (J. Williams), SAIC (J. Zurer), Walter Reed Army Medical Center (P. Peller), Johns Hopkins Medical Institutions (M. Murrell and J. Leal), and Maryland Psychiatric Research Center (M. Zhao). Additionally, we thank Dr Karl Friston (Wellcome Department of Cognitive Neurology, London) for Statistical Parametric Mapping software.

This study was done in partial fulfillment of the first author's doctoral degree in Applied-Experimental Psychology at George Mason University, Fairfax, Virginia (Advisor: R. Smith. Committee Members: R. Holt and D. Boehm-Davis, Department of Psychology; and H. Morowitz, Krasnow Institute for Advanced Study).

#### DEPARTMENT OF DEFENSE DISCLAIMER

Human volunteers participated in this study after giving their free and informed consent. Investigators adhered to AR 70–25 and USAMRDC Reg 70–50 on the use of volunteers in research. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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