Decreased Cortical Response to Verbal Working Memory Following Sleep Deprivation

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Study Objective: To investigate the cerebral hemodynamic response to verbal working memory following sleep deprivation.

Design: Subjects were scheduled for 3 functional magnetic resonance imaging scanning visits: an initial screening day (screening state), after a normal night of sleep (rested state), and after 30 hours of sleep deprivation (sleep-deprivation state). Subjects performed the Sternberg working memory task alternated with a control task during an approximate 13-minute functional magnetic resonance imaging scan.

Setting: Inpatient General Clinical Research Center and outpatient functional magnetic resonance imaging center.

Patients or Participants: Results from 33 men (mean age, 28.6 ± 6.6 years) were included in the final analyses.

Interventions: None.

Measurements and Results: Subjects performed the same Sternberg working memory task at the 3 states within the magnetic resonance imaging scanner. Neuroimaging data revealed that, in the screening and rested states, the brain regions activated by the Sternberg working memory task were found in the left dorsolateral prefrontal cortex, Broca's area, supplementary motor area, right ventrolateral prefrontal cortex, and the bilateral posterior parietal cortexes. After 30 hours of sleep deprivation, the activations in these brain regions significantly decreased, especially in the bilateral posterior parietal cortices. Task performance also decreased. A repeated-measures analysis of variance revealed that subjects at the screening and rested states had similar activation patterns, with each having significantly more activation than during the sleep-deprivation state.

Conclusions: These results suggest that human sleep-deprivation deficits are not caused solely or even predominantly by prefrontal cortex dysfunction and that the parietal cortex, in particular, and other brain regions involved in verbal working memory exhibit significant sleep-deprivation vulnerability.

Key words: verbal working memory, sleep deprivation, functional magnetic resonance imaging.

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INTRODUCTION

SLEEP DEPRIVATION IN HUMANS CAUSES PERFORMANCE DEFICITS ON A VARIETY OF COGNITIVE TASKS (FOR REVIEWS, SEE HARRISON AND HORNE[1] AND PILCHER AND HUFFCUTT[2]). Our society is increasingly sleep deprived, and many jobs, such as those of pilots, military personnel, and medical workers, require people to function while they are sleep deprived, at least temporarily. It is therefore important to better understand how sleep deprivation impairs cognitive performance, both in terms of behavior and the neurobiologic mechanisms involved. In terms of the brain regions affected by sleep deprivation, much early attention has focused on the prefrontal cortex (PFC) as being particularly vulnerable to sleep deprivation.[1-4] It is unclear whether sleep-deprivation deficits arise from dysfunction of the PFC alone or whether other brain regions are involved.

Functional neuroimaging techniques now allow investigators to directly investigate brain responses following sleep deprivation with normal healthy subjects.[5-11] To date, only a few published functional imaging studies have investigated the human brain response to working memory with sleep-deprived subjects using arithmetic working-memory tasks during either functional magnetic resonance imaging (fMRI)[8] or positron emission tomography.[10] These studies supported Horne's PFC vulnerability hypothesis,[3] which proposes that the PFC is particularly vulnerable to sleep deprivation, and that performance deficits following sleep deprivation arise from PFC dysfunction. In contrast to arithmetic working memory, verbal working memory has been demonstrated to play a significant role in language comprehension and problem solving. Its functional anatomy has been well established using neuroimaging approaches.[12-14] However, little is known about the functional cortical response to verbal working-memory demands in the human brain following sleep deprivation. Recently, Drummond et al found that, during the Baddeley's logical reasoning task, sleep deprivation led to significant increases in brain activation in several regions, including the left PFC and bilateral inferior parietal cortexes.[15] The Sternberg Working Memory Task (SWMT)[16] has been widely used as a verbal working-memory task[17-19] and shows changes following sleep deprivation.[20,21] In order to formally test whether the PFC is selectively vulnerable to sleep deprivation (Horne's hypothesis), we used fMRI while subjects performed the SWMT and investigated the hemodynamic response to verbal working memory both globally and regionally following 30 hours of sleep deprivation.

We hypothesized that sleep deprivation would result in significantly reduced activation, both globally and regionally, in selective brain regions involved in verbal working memory. These

Disclosure Statement

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specific regions include the PFC, especially the dorsolateral PFC, which manipulates and maintains working-memory information[12-14,17-19]; the posterior parietal cortex (PPC), which mediates verbal storage[12,14,22]; and a set of anterior speech regions (Broca's area, supplementary motor area (SMA) and premotor area (PMA), which mediate verbal rehearsal.[12,14]

METHODS

Subjects and Behavioral Control

Thirty-three healthy men between the ages of 18 and 45 years (mean 28.6 ± 6.6 years; education, mean 16.2 ± 2.6 years), with no history of medical, neurological, psychiatric, or sleep disorders, were recruited and participated in the following fMRI study approved by Medical University of South Carolina Institutional Review Board after providing a written informed consent. Subjects who abused alcohol or drugs were excluded. They habitually maintained normal sleep schedules of 7 to 9 hours per night, between 10:00 PM and 8:00 AM. During the study, subjects followed their regular sleep schedules. Sleep deprivation was performed from Wednesday beginning at 3:00 AM to Thursday at 9:00 AM, where the subjects were checked every 10 minutes to make sure that they were awake (1 night of partial sleep deprivation followed by a full day and night of sleep loss, for a total of 30 hours of sleep deprivation). Subjects were not allowed any caffeine or alcohol throughout the week. Caffeine-free meals were provided during the sleep-deprivation period.

fMRI Scanning and Data Analysis

Subjects were scheduled for 3 fMRI scanning visits: an initial screening day (screening state) on Monday or Thursday, after a normal night of sleep (rested state) on Monday, and after 30 hours of sleep deprivation (sleep-deprivation state) on Thursday. Initial scans occurred at 9 AM to eliminate possible circadian-confound effects. Subjects performed the SWMT while being scanned with fMRI in the following order: (1) the screening state, (2) the rested state, and (3) the sleep-deprivation state (Figure 1a). This scan order was consistent for all subjects, and we did not use a counterbalanced order due to our particular design. We instead employed a repeated-measures scanning to clarify potential within-subjects confounds. To minimize the potential task practice effects, first, before the first screening scanning sessions, all the subjects had practiced the SWMT 10 times, and they all had no improved performance with practice. The practice effects over time are presented in Figure 1b. A repeated-measures analysis of variance (ANOVA) showed a significant decrease in reaction time with the first 4 times of practice ($F_{3,96} = 37.30$, $P < .0001$), posthoc t tests revealed significant decreases between the first and the second times ($P < .0001$), as well as between the second and the third times ($P < .001$). There was no significant difference between the third and the fourth times ($P > .2$). Subjects reached a stable (asymptotic) level after 4 times of practice, without significant change in performance over practice. Second, we directly compared both the performance and brain activation between the screening state and rested state to investigate poten-

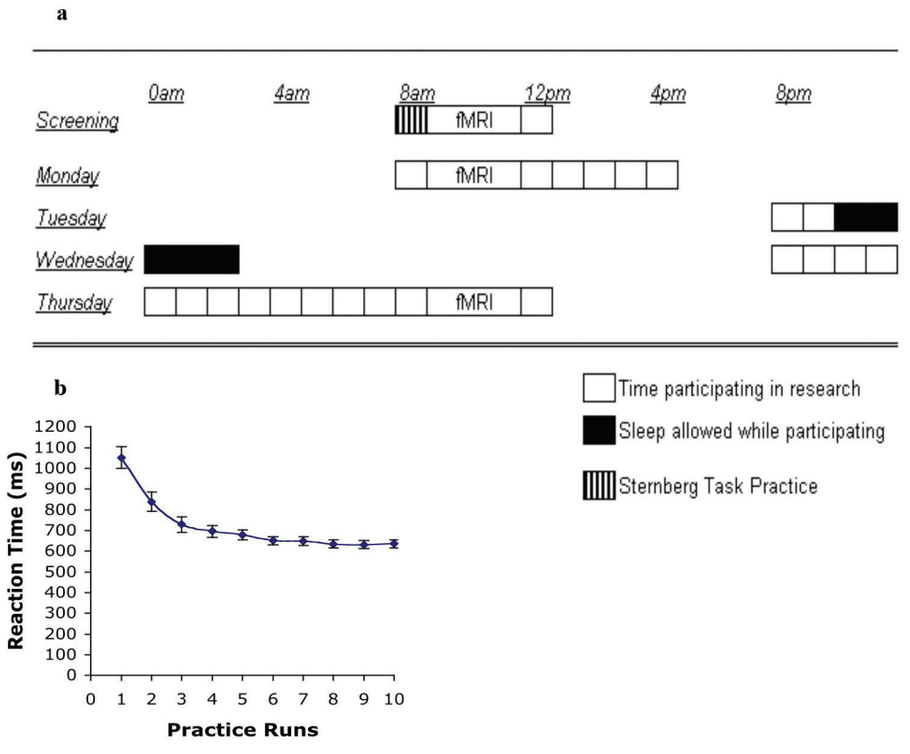

Figure 1a—Functional magnetic resonance imaging (fMRI) scanning protocol. Subjects were scheduled for MRI scanning 3 times: screening (Monday or Thursday), after a normal night of sleep (Monday), and after 30 hours of sleep deprivation (Thursday). Sleep deprivation time was from Wednesday at 3:00 am through Thursday at 9:00 am.

Figure 1b—The Sternberg Task: practice effects with time. Significant decreases in reaction times were seen with the first 4 times of practice, and subjects reached an asymptotic performance level after 4 times of practice. Error bars represent SEM. Prior to the screening scan sessions, subjects had practiced the Sternberg task 10 times.

tial task-practice effects between these 2 states and then applied it to the rested state and the sleep-deprivation state. This scanning was done as a part of a larger program designed to determine whether image-guided transcranial magnetic stimulation (TMS) might be able to improve performance following sleep deprivation. Thus, 24 of these subjects each had 49 minutes of TMS on Wednesday morning at 10:00, with 9 subjects receiving sham TMS. Comparisons were done between those who received active versus sham TMS, and there was no difference; thus, overall results are presented for all 33 subjects (see behavioral and fMRI results below for details).

The SWMT performed during the fMRI scan was modified to fit an imaging design and to be able to acquire behavioral data within the scanner. Briefly, each functional scan consisted of 12 blocks. Each block included a control task (32 seconds) with an alternative Sternberg task (32 seconds), starting from control task. Each task contained 2 trials. Each trial lasted for 16 seconds. The entire functional scan lasted for 12 minutes, 48 seconds. An Integrated Functional Imaging System (IFIS) (Gainesville, FL) was used to display the letters and asterisks that allowed subjects to view the stimuli on an LCD screen in front of their eyes. Subjects were instructed to hold 2 hand pads with their hands and respond with their thumbs (left or right) to *YES* or *NO*, randomized between individuals. The control trial consisted of a 3-second viewing of 6 asterisks in 2 rows, followed by a 7-second delay, and then a 3-second viewing of a word *YES* or *NO* presented at the center of the screen. During the control trial, each subject was asked to press the appropriate button for *Yes* or *No* when *YES* or *NO* was presented on the IFIS screen at a randomized order. During the Sternberg trial, arrays of either 1, 3, or 6 letters were randomized to display on the IFIS screen. Subjects viewed the set of letters for 3 seconds (recognition). They then maintained this set in mind across a 7-second delay (retention). Subsequently, a probe letter was presented on the screen for 3 seconds, and subjects responded *yes* or *no* according to whether the probe letter had been included in the previously viewed set (recall). There was a 1.5-second time-out interval (rest) after the probe letter was presented and another 1.5-second time-out interval before the display of the "recognition" letter or letters (Figure 2). Subjects were instructed to respond as accurately as possible. Reaction times and the number of errors were recorded within IFIS. Behavioral data were not adequately stored for some subjects due to mechanical malfunctions, leaving data from only 30 subjects available for the behavioral data analysis between the rested state and sleep-deprivation state and data from 15 subjects available between the screening state and rested state. All images were acquired with a 3T MRI scanner (Intera, Philips Medical System, The Netherlands) using a send/receive single-channel head coil. A set of T_1-weighted axial structural images encompassing the whole brain was acquired using the following parameters: TR = 625 milliseconds, TE = 20 milliseconds, slice thickness = 5 mm, gap = 1 mm, field of view (FOV) = 25.6 cm, number of slices = 24, matrix = 256 × 256. With the same slice coverage as with the structural scans, a whole-brain gradient echoplanar-imaging sequence was employed to acquire continuously on 24 slices in an ascending fashion in the axial plane for each functional scan; the parameters used were TR = 2670 milliseconds, TE = 40 milliseconds, FOV = 25.6 cm, image matrix = 64 × 64, in-plane pixel size = 4 × 4 mm, slice thickness = 5 mm, and gap = 1 mm. One hundred-sixty 1-mm contiguous axial high-resolution anatomic images were also acquired for each subject (256 × 256 matrix, FOV = 25.6 cm). At the rested state and sleep-deprivation state, the scan data from all of the 33 subjects were acquired with IFIS; at the screening state, the data from 22 subjects were acquired with IFIS, and the other 11 subjects using a projector and back-projection system (prior to the availability of the IFIS). The same e-prime script was used for all scans, with stimulus display times remaining constant. After scanning at the rested and sleep-deprivation states, subjects completed sleepiness-related visual analogue scales and Epworth Sleepiness Scales.[23]

Functional-image analysis was conducted using Statistical Parametric Mapping software (SPM 2, Wellcome Department of Cognitive Neurology, London, UK). Echoplanar imaging scans were corrected for motion and coregistered to the T_1-weighted structural images. After motion correction, all functional scans had residual motion movement less than 1 mm in any of the 3 planes and were thus included for further analysis. The functional images were then spatially normalized to the SPM template and resampled with a voxel size of 2 × 2 × 2 mm.[24] After normalization, functional images were spatially smoothed using a Gaussian kernel with 6-mm full width at half maximum to condition for random field theory, which was applied to correct for multiple comparisons in statistical parametric mapping.[25] For creating individual t-maps, the block design was convolved with a hemodynamic response function that approximated the activation patterns. Effects at each voxel were estimated using the general linear model at the first statistical level. A box-car reference function modeled the activation blocks. The motion-recorded parameters generated during the "realign" process were applied to reject the motion-related activation as 6 user-specified regressors. A high-pass filter (cut-off frequency = 128 seconds) was used to remove possible effects of low-frequency changes. The individual activated and deactivated t-maps were generated by defining the contrasts of the Sternberg task vs the control task and the control task vs the Sternberg task, respectively. Group analyses focused on 2 issues. First, areas of significant activation were identified separately for each state (screening, rested, sleep deprivation). Second, the rested and sleep-deprivation states were directly compared to identify regions that were significantly more responsive to the SWMT after 1 state compared to the other. For the within-state analysis, 33 individual contrast images generated at the first statistical level were then used to create a group t-map in a random-

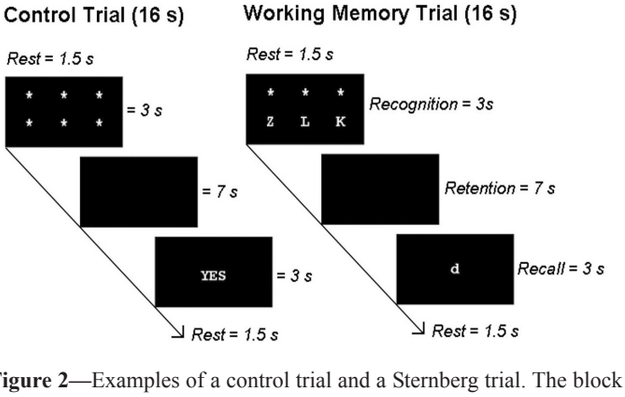

Figure 2—Examples of a control trial and a Sternberg trial. The block design consisted of 12 blocks each containing a control task (32 seconds) with an alternative Sternberg task (32 seconds). Each task consisted of 2 trials.

effects model[26] for each state; cluster analyses were performed at the same threshold of $P_{corrected} < .005$ for each state's group map. Based on the results of cluster analyses, activated voxels that passed the threshold of $P_{corrected} < .005$ were identified from individual t-maps for each activated brain region at each state. Time courses were also individually extracted for all activated voxels that passed the threshold of $P_{corrected} < .005$ in each activated region (except the right parietal cortex because there were no significant activated voxels at this threshold after sleep deprivation; the threshold used in this region was $P_{corrected} < .05$), and first averaged across cycles within scan and then converted to percentage of signal change (PSC) for each functional scan.[27] Employing Statistical Package for Social Science (SPSS 10.0 for Macintosh, SPSS, Inc., Chicago, Ill), the number of activated voxels (NAV) was compared with paired-sample *t* tests. During the Sternberg task, time-course activations expressed as PSC[27,28] were also compared at global and regional levels using a repeated-measures ANOVA. For the between-states analysis, all individual contrast images were input to a paired *t* test model in SPM 2 to generate the difference group t-map. Cluster analysis was also performed a priori with a cutoff of uncorrected $P < .01$ with a spatial extent of $P < .05$ (corrected for multiple comparisons).[29] After this analysis was complete, considering that use of suprathreshold voxels possibly limits the inferences concerning sleep-deprivation effects, we applied a lower threshold of uncorrected $P < .001$ to the within-state analysis and of uncorrected $P < .05$ to the between-states analysis to check whether the effects of sleep deprivation exhibited consistent or similar patterns. Several data analyses were done to clarify whether TMS during the first day of sleep deprivation affected the sleep-deprivation scan on the following morning. First, NAV and PSC, which were extracted from individual t-maps and echoplanar-imaging data at the identical threshold $P_{corrected} < 0.005$, were used to analyze potential TMS effects and its interaction effects with sleep deprivation in a general linear model. Second, an analysis of covariance was also conducted between the rested state and the sleep-deprivation state in 33 subjects by treating the TMS as a covariable to generate a difference map. This map was compared with another difference map also generated using an analysis of covariance model that did not consider the TMS as a covariable. In order to clarify whether the additional practice of the SWMT over the 3 scanning sessions significantly affected brain activation, we performed the following analyses: (1) A repeatability test of global activation (NAV) using a repeat-measures ANOVA. Because this model requires equal sample sizes, and 22 subjects each had the 3 scans across the 3 states, we used the data from the 22 subjects to investigate the repeatability in the brain activation. This was done with SPSS. (2) Direction maps comparisons. Using the data from the 22 subjects with SPM 2, we specified a within-subjects ANOVA model and created maps between each pair of the 3 states (rested vs sleep deprivation, rested vs screening, and screening vs sleep deprivation). (3) Performance comparisons between the screening state and rested state, including reaction times and number of errors. This was also done with SPSS. Finally, we wondered whether sleep deprivation produced task difficulty related responses in our current study. Treating the 1-letter, 3-letter, 6-letter, and control tasks as 4 separate conditions, and defining contrasts of 1-letter vs control, 3-letter vs control, and 6-letter vs control, individual t-maps were generated by specifying and estimating the general linear model in SPM2. Similarly, the opposite contrast t-maps were generated in the same way by defining contrasts of control vs 1-letter, control vs 3-letter, and control vs 6-letter tasks. Based on these contrast images, group maps were separately created at the second level using 1-sample *t* tests. Cluster analyses were carried out, and there were large differences in brain activation induced by 3 types of letter tasks. For comparison, we selected an uncorrected $P < .001$ with a spatial extent $P < .05$ (corrected for multiple comparisons) to define significant brain activation and deactivation in the within-state clusters analyses. Paired *t*-test models were also employed to generate maps showing differences between the normal sleep and sleep deprivation during the 1-letter, 3-letter, and 6-letter tasks. An uncorrected $P < .01$ with a spatial extent of $P < .05$ (corrected for multiple comparisons) was used for the between-states clusters analyses.

RESULTS

Behavioral Results

Since 15 subjects each had available behavioral data from the IFIS across the 3 states, we conducted a repeat-measures ANOVA within these subjects and found that sleep deprivation induced a significant overall performance decrement with slower reaction times ($F_{2,28} = 5.16$, $P < .05$) and more errors ($F_{2,28} = 6.58$, $P < .005$). Posthoc comparisons revealed that, from the initial screening state to the rested state, there was no significant change in performance (reaction times: 600 ± 96 milliseconds vs 621 ± 96 milliseconds, $P > .05$, screening state vs rested state; number of errors: 2.1 ± 1.2 vs 1.7 ± 1.3, $P > .05$, screening state vs rested state). In contrast, however, following 30 hours of sleep deprivation compared to the other 2 states, while performing the SWMT within the scanner, subjects had significantly longer reaction times (600 ± 96 milliseconds vs 707 ± 187 milliseconds, $P < .05$, screening state vs sleep-deprived state; 621 ± 96 milliseconds vs 707 ± 187 milliseconds, $P < .05$, rested state vs sleep-deprived state) and more errors (2.1 ± 1.2 vs 3.3 ± 1.8, $P < .01$, screening state vs sleep-deprived state; 1.7 ± 1.3 vs 3.3 ± 1.8, $P < .01$, rested state vs sleep-deprived state). Since 30 subjects each had available behavioral data from the IFIS between the rested state and sleep-deprivation state, we also performed a paired-sample *t* test with this larger sample size and found that sleep deprivation also significantly led to slower reaction times (727 ± 213 milliseconds vs 795 ± 222 milliseconds, $t_{29} = 2.14$, $P < .05$, rested state vs sleep-deprived state) and more errors (1.4 ± 1.4 vs 3.0 ± 1.7, $t_{29} = 4.27$, $P < .0001$ rested state vs sleep-deprived state). A repeated-measures ANOVA revealed that, at the sleep-deprivation state, subjects had significantly longer reaction times as the task difficulty increased ($F_{2,58} = 12.99$, $P < .0001$); posthoc comparisons indicated that there was no significant difference between the 1-letter and 3-letter tasks ($P > .05$), but both the 1-letter and 3-letter tasks had significantly shorter reaction times than the 6-letter task ($P < .0001$, $P < .05$ respectively). Although a repeated-measures ANOVA also revealed a significant overall effects in number of errors across 3 types of letter tasks ($F_{2,58} = 13.64$, $P < .0001$) following sleep deprivation, posthoc *t* tests showed a nonsignificant difference between the 1-letter and 6-letter tasks ($P > .3$), and both the 1-letter and 6-letter tasks had significantly more errors than the 3-letter task ($P < .0001$ respectively). That is, subjects did not have more errors with the

increased task difficulties. In addition, sleep deprivation induced significant increases in the Epworth Sleepiness Scale (6.54 ± 3.76 vs 16.60 ± 6.66, $P < .0001$, rested state vs sleep-deprived state) and in the visual analog scale (2.33 ± 1.77 vs 8.47 ± 1.37, $P < .0001$, rested state vs sleep-deprived state), tested using paired *t* tests. In order to understand whether TMS produced potential effects on subjects' performance as well as its interaction with sleep deprivation, reaction times and error rates collected at the Bain Stimulation Laboratory before and after sleep deprivation were analyzed using a general linear model. Treating the reaction times as a dependent variable, TMS as a between-subjects factor, and sleep deprivation as a within-subjects factor, a general linear model revealed that there was no significant TMS effects on reaction times ($F_{1,31} = 1.46$, $P > .2$) and interaction effects with sleep deprivation ($F_{1,31} = 2.15$, $P > .1$). Similarly, treating the error rates as a dependent variable, TMS as a between-subjects factor, and sleep deprivation as a within-subjects factor, a general linear model also revealed that there was no significant TMS effects on error rates ($F_{1,31} = 0.003$, $P > .9$ as well as interaction effects with sleep deprivation ($F_{1,31} = 0.14$, $P > .7$). A comprehensive manuscript specifically regarding the absence of TMS effects on sleep deprivation is being prepared and will be submitted elsewhere.

fMRI Results

Identification of Activated Brain Regions for Each State (Within-State Analysis)

At the screening and the rested states, performing the SWMT task compared to the control task resulted in significant activation in brain regions involved in verbal working memory, including the left dorsolateral PFC (Brodmann areas (BA) 9, 45, 46), left PPC (BA 7, 40), left Broca's area (BA 44), left SMA (the activation in left SMA, extended partly to right SMA (BA 6), left PMA (BA 6), bilateral anterior cingulate gyri (BA 32)), right inferior PPC (BA 40), and right ventrolateral PFC (primarily in the inferior frontal gyrus, BA 44) (Figure 3a). After 30 hours of sleep deprivation during the sleep-deprivation state, the activations in the above areas were markedly reduced, especially in the bilateral PPC (all the right parietal activation and most of the left parietal activation was no longer seen following sleep deprivation) (Figure 3b). No new activated regions were found after sleep deprivation compared to the rested state. All the results are significant at the identical threshold $P < .005$ (voxel level and cluster level corrected for multiple comparisons) ($P_{corrected} < .005$). Note that the activation in the left hemisphere was more than that in the right hemisphere. No deactivated (control > Sternberg) areas were found at this strict threshold in either the

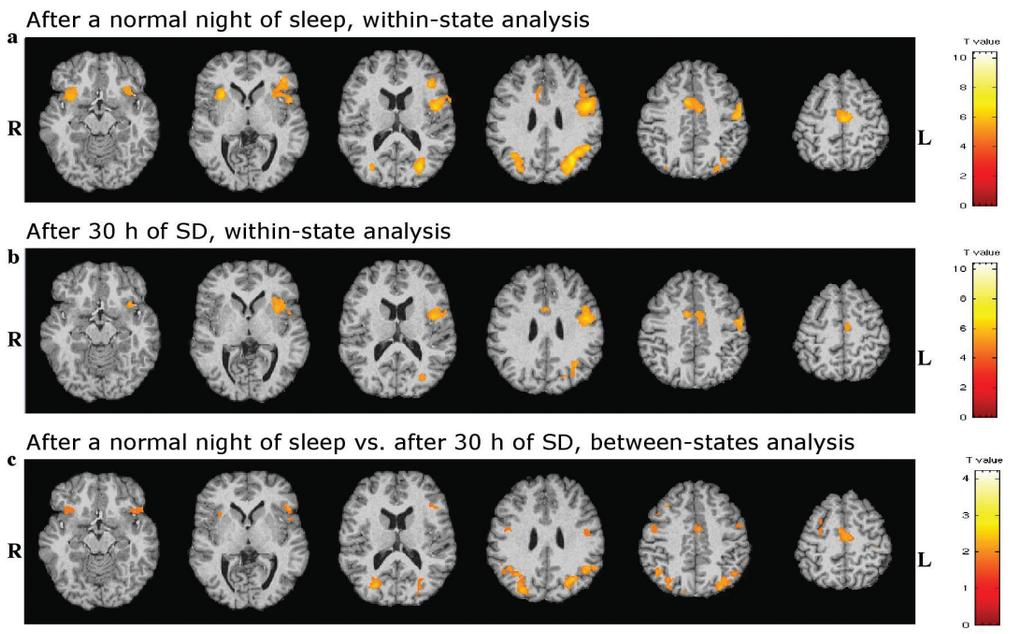

Figure 3—Significant brain activation identified by within-state analysis and between-states analysis. Maps are displayed at 6 different brain levels (from 16 mm below to 64 mm above the bicommissural plane). a. After a normal night of sleep, within-state analysis. Map was thresholded at P < .005 (corrected for multiple comparisons). Significant activation (Sternberg task > control) was found in bilateral posterior parietal cortex (PPC), left dorsolateral prefrontal cortex (PFC), left Broca's area, and left supplementary motor area (SMA) (partly involved in right SMA, left premotor area (PMA), and bilateral anterior cingulate gyri), and right ventrolateral PFC. No deactivation (control > Sternberg task) was found. Note that the right dorsolateral PFC was not activated at this threshold; however, it was significantly activated when the threshold was lowered to uncorrected P < .001 with a spatial extent of P < .05 (corrected for multiple comparisons). b. After 30 hours of sleep deprivation (SD), within-state analysis. Map was thresholded at P < .005 (corrected for multiple comparisons). Significant activation was only found in left dorsolateral PFC, Broca's area, SMA, and inferior parietal cortex. No deactivation (control > Sternberg task) was found. c. Between the rested and SD states analysis. Map was thresholded at P < .01 with a spatial extent of P < .05 (corrected for multiple comparisons). Significant difference in activation (normal sleep > SD) was found in bilateral dorsolateral PFC, PPC, SMA, and PMA, left Broca's area, and right ventrolateral PFC. No deactivation (SD > normal sleep) was found. Note that there are more activations (number of activated voxels) differences in the bilateral parietal cortices than other regions. Also note that the right dorsolateral PFC and PMA show significant differences in regional activation, which were not significant at the map after a normal night of sleep at the threshold of corrected P < .005.

group map after a normal night of sleep or the group map after sleep deprivation. When a lower threshold (uncorrected $P < .001$) was applied to the cluster analysis within each state, more activated voxels were included in the maps before and after sleep deprivation; however, similar decreased changes induced by sleep deprivation were also observed.

Identification of Activated Brain Regions Between the Rested and Sleep-deprivation States

A paired-sample *t*-test map showed that brain regions involved in verbal working memory had significantly more activation after a normal night of sleep than after sleep deprivation, including the left dorsolateral PFC (BA 9, 45, 46), left PPC (BA 7, 40), and left Broca's area (BA 44), left SMA (including right SMA, left PMA (BA 6) and bilateral anterior cingulate gyri (BA 32)), right dorsolateral PFC (BA 45, 46), right PPC (BA 7, 40), right ventrolateral PFC (BA 44), and right PMA (Figure 3c). An a priori threshold of $P < .01$ with a spatial extent of $P < .05$ (corrected for multiple comparisons) was set to define significant activation in the between-states analysis. There were no activated regions that had significantly more activation at the sleep-deprivation state than at the rested state. Table 1 lists the cluster-analysis results of brain regions activated by SWMT within and between the rested and sleep-deprivation states. When the threshold was lowered to an uncorrected value of $P < .05$, significant differences were also noted in these areas with more activated voxels, with the same overall findings remaining unchanged.

Testing of Global Activations

Compared to the rested state, sleep deprivation induced a significant global decrease, as measured by the NAV ($t_{32} = 4.88$, $P < .0001$, 2-tailed paired *t* test) (Figure 4a) and by the PSC ($F_{1,32} = 10.50$, $P < .003$, tested with a repeated-measures ANOVA treating the sleep-deprived (sleep-deprivation state) and non–sleep-deprived (rested baseline state) condition as a within-subjects factor) (Figure 4b) (see the legend of the Figure 4b for information about the explanation of cycle-averaged time curves, includ-

Table 1—Regions of Significant Cortical Activation and Deactivation Following Normal Sleep and Sleep Deprivation in 33 Men

Brain region	BA	MNI coordinates (mm) X	Y	Z	Cluster size Number of voxels	T value
Normal sleep (within-state analysis)						
Activated (SWMT >Control)						
Left dorsolateral PFC	9,45,46	-48	2	24	3023	9.47
Left posterior parietal cortex	7,40	-30	-76	20	1953	10.47
Left Broca's area	44	-44	36	6	552	7.63
Left SMA and premotor area	6	-2	-14	58	1227	8.43
Right ventrolateral PFC	44	34	14	-2	704	8.34
Right posterior parietal cortex	40	30	-78	22	755	7.68
Deactivated (Control > SWMT)						
None						
Sleep deprivation (within-state analysis)						
Activated (SWMT > Control)						
Left dorsolateral PFC	9,45,46	-50	0	32	1494	7.68
Left Broca's area	44	-38	18	-6	351	7.40
Left posterior parietal cortex	40	-36	-58	28	345	5.82
Left SMA and premotor area	6	-8	4	46	513	7.08
Right ventrolateral PFC	44	30	12	2	74	5.32
Deactivated (Control > SWMT)						
None						
Normal sleep>sleep deprivation (between-states analysis)						
Left dorsolateral PFC	9,45,46	-52	2	38	139	3.95
Left posterior parietal cortex	7,40	-32	-70	32	699	4.24
Left Broca's area	44	-40	26	-22	56	3.25
Left SMA and premotor area	6	-4	-2	64	205	3.86
Right ventrolateral PFC	44	40	28	-22	35	3.38
Right posterior parietal cortex	7,40	26	-76	20	614	4.48
Right dorsolateral PFC	9,45,46	48	-2	22	69	3.71
Right premotor area	6	32	-4	64	46	3.03
Sleep deprivation >normal sleep (between-states analysis)						
None						

Scans were obtained after normal sleep or 30 hours of sleep deprivation. The identical threshold for cluster analysis was $P < .005$ (false discovery rate corrected for multiple comparisons) in the within-state analyses and an uncorrected $P < 0.01$ with a spatial extent of $P < 0.05$ (corrected for multiple comparisons) in the between-states analysis. MNI refers to Montreal Neurological Institute; BA, Brodmann area; SWMT, Sternberg Working Memory Task; PFC, prefrontal cortex; SMA: supplementary motor area. Voxel size = 2 × 2 × 2.

ing hemodynamic response delay[28,30] and poststimulus undershoot[31]). Interestingly, a repeated-measures ANOVA also revealed a significant decrease in PSC during the control task following sleep deprivation ($F_{1,32} = 24.21$, $P < .0001$).

Testing of Regional Activations

Following sleep deprivation, a paired *t* test also revealed a significant NAV decrease of regional activation in the left dorsolateral PFC ($t_{32} = 3.97$, $P < .0001$), left PPC ($t_{32} = 5.41$, $P < .0001$), left Broca's area ($t_{32} = 2.56$, $P < .05$), left SMA and PMA ($t_{32} = 3.20$, $P < .003$), right PPC (the parietal activation dropped off after sleep deprivation, and there were no activated voxels in any individual subjects), and right ventrolateral PFC ($t_{32} = 3.04$, $P < .005$) (Figure 5a, c, e, g, i, and k). Treating the sleep-deprived (sleep-deprivation) and non–sleep-deprived (rested baseline) condition as a within-subjects factor, a repeated-measures ANOVA revealed a significant overall reduction in the PSC in the left PPC ($F_{1,32} = 27.59$, $P < .0001$), left Broca's area ($F_{1,32} = 7.79$, $P < .009$), left SMA and PMA ($F_{1,32} = 20.12$, $P < .0001$), right PPC ($F_{1,32} = 76.33$, $P < .0001$), and right ventrolateral PFC ($F_{1,32} = 19.51$, $P < .0001$) (Figure 5d, f, h, j, and l). However, there was no significant change of the PSC in the left dorsolateral PFC ($F_{1,32} = 2.62$, $p = 0.11$) following sleep deprivation (Figure 5b). The left dorsolateral PFC showed a sustained time-curve activation with a relatively shorter hemodynamic delay (6 seconds). The left parietal cortex exhibited a similar time course pattern to the right parietal cortex, with a relatively shorter hemodynamic delay (6 seconds). The left Broca's area, SMA, and PMA, as well as the right ventrolateral PFC, showed similar time-course patterns with relatively longer hemodynamic delay (6-13 seconds). These differentiable patterns of hemodynamic responses are presumably associated with the role that they play during verbal working memory. Of these time courses after sleep deprivation, the amplitudes of the signal were reduced, but the shapes (patterns) of time curves remained similar relative to those before sleep deprivation.

Clarification of Potential TMS Effects and its Interaction with Sleep Deprivation

After extracting the NAV and PSC (at an identical statistical threshold ($P < .005$, corrected for multiple comparisons) for each individual subject, treating the global NAV as a dependent variable, TMS as a between-subjects factor, and sleep deprivation as a within-subjects factor, a general linear model revealed nonsignificant TMS effects ($F_{1,31} = 0.005$, $P > .9$) on NAV and its interaction effects with sleep deprivation ($F_{1,31} = 1.33$, $P > .7$). Similarly, treating the global PSC as another dependent variable, a general linear model also revealed nonsignificant TMS effects on PSC ($F_{1,31} = 1.04$, $P > .3$) and interaction effects with sleep deprivation ($F_{1,31} = 0.27$, $P > .8$). These were done in SPSS. In addition, the analysis of covariance model in SPM 2 also indicated that there was no sig-

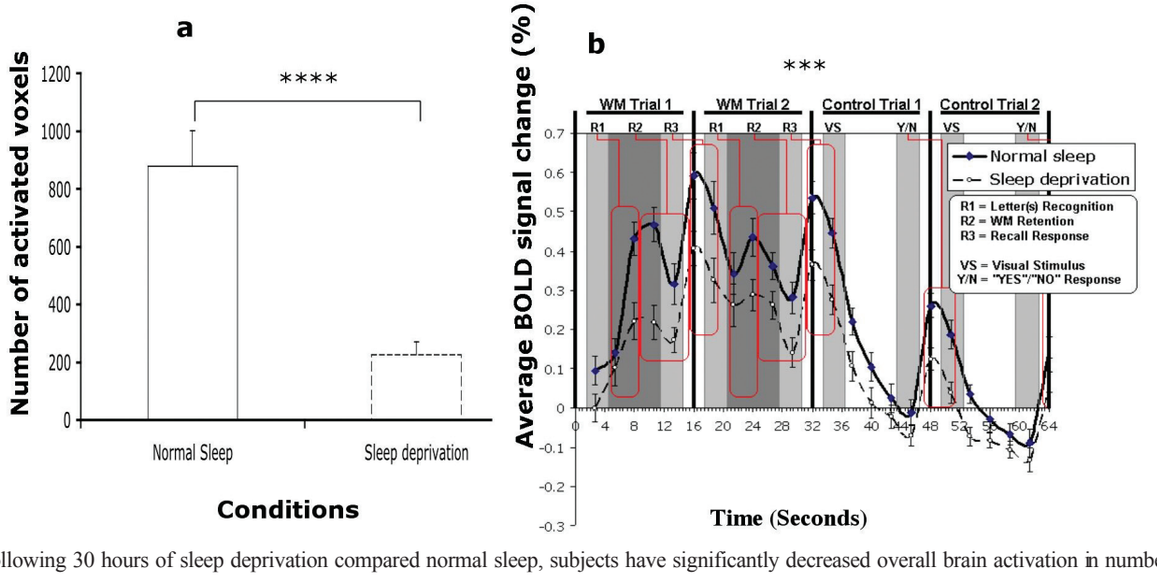

Figure 4—Following 30 hours of sleep deprivation compared normal sleep, subjects have significantly decreased overall brain activation in number of activated voxels (a) and percentage of signal change (b) (n = 33). a: Sleep deprivation led to a significant decrease of global activation in number of activated voxels. b: Cycle-averaged hemodynamic time-curves during Sternberg task show significant decrease in the percentage of signal change following sleep deprivation. The solid line represents the condition of normal sleep, and the dashed line represents the condition of sleep deprivation. Error bars represent SEM. ***P < .003, ****P < .0001, paired 2-tailed t tests. The averaged cycle includes a 32-second Sternberg working memory (WM) task (2 trials each lasting for 16 seconds) alternated with a 32-second control task (also 2 trials each lasting for 16 seconds). (See Figure 2 for subcomponent timing of each trial). However, because of the hemodynamic delay (approximately 6 seconds, see Newman et al[28] and Bandettini et al[30]), the hemodynamic response curves to Sternberg task were shifted to 6-38 seconds (as indicated by the rectangles offset from the subcomponents of paradigm presentation, which show very similar time-curve patterns of the 2 Sternberg trials) from 1-32 seconds. The period 1-6 seconds represent the hemodynamic delay. The period 6-22 seconds represent the response to the first Sternberg trial. 6-7.5 seconds: rest, 7.5-10.5 seconds: recognition, 10.5-17.5 seconds: retention, 17.5-20.5 seconds: recall, 20.5-22 seconds: rest. The time period 22 - 38 seconds represent the response to the second Sternberg trial. The 22-23.5 seconds: rest, 23.5-26.5 seconds : recognition, 26.5-33.5 seconds: retention, 33.5-36.5 seconds: recall, 36.5-38 seconds: rest. Note that there are 2 relatively weaker peak activations (occurred at 48 seconds and 64 seconds when subjects answered Yes or No question) during the control trial 1 and trial 2 that corresponded to the peak response (occurred at 16 seconds and 32 seconds) during the WM trial 1 and trial 2. These peak activations involved in potential memory scanning, binary decision, response selection, attention, motor output process, and visual component (see method section). There are 2 poststimulus undershoots in the control trials (see Jasdzewski et al[31]). BOLD refers to blood oxygenation level dependent.

nificant difference between maps that considered TMS as a covariable and maps that did not consider TMS as a covariable.

Clarification of Possible Confounds Across Scan Sessions Within Subjects.

Treating the NAV as a dependent variable and scanning (screening state, rested state, and sleep-deprivation state) as a within-subjects factor, a repeated-measures ANOVA of global activations at a common statistical threshold ($P < .05$, corrected for multiple comparisons) revealed a significant overall effect on NAV across the 3 sessions ($F_{2,42} = 9.28$, $P < .0001$). Posthoc t tests revealed that the screening state and the rested state each had significantly more activation than the sleep-deprivation state ($P < .005$ and $P < .0001$ respectively). There was no significant difference in global NAV between the screening state and the rested state ($P > .46$) (Figure 6). The t-map comparison between the screening and rested states also indicated there was no significant difference. Given that the task was relatively long, we divided the scan into 3 parts with equal lengths and checked both the performance and time-course activation at the beginning, middle, and end of the scanning at the rested state, and we did not find time-on-task effects. Similarly, we also analyzed both the performance and time-course activation at the beginning, middle, and end of the scanning at the sleep-deprivation state, and we failed to find any significant differences among the 3 parts, suggesting that sleep-deprived subjects did not significantly change their approach or attitude to the Sternberg task.

Task Difficulty-related Brain Responses Following Normal Sleep and Sleep Deprivation

Following normal sleep, all the 3 types of letter tasks predominately induced significant brain activation in the left frontoparietal circuits. As the task difficulty increased, more activation was observed. There existed a substantial difference between the 6-letter and the 1-letter or 3-letter tasks, and there was no obvious difference in activation between the 1-letter and 3-letter tasks. Following sleep deprivation, all the activation seen under normal sleep condition exhibited great decreases, without any newly activated brain regions generated. Similarly to the brain response with the task difficulties at the rested condition, more responses were seen during the 6-letter tasks relative to 1-letter or 3-letter tasks. The results of cluster analyses on the 1-letter, 3-letter, and 6-letter Sternberg tasks are presented in Table 2, and 3-D projections of glass brains following normal sleep and sleep deprivation, as well as related paired-sample t test comparison maps, are displayed in figure 7.

Correlations Between Task Performance and Cerebral Activation

Examining the relationship between the behavior and brain activity in subjects during the sleep-deprivation state scan, reac-

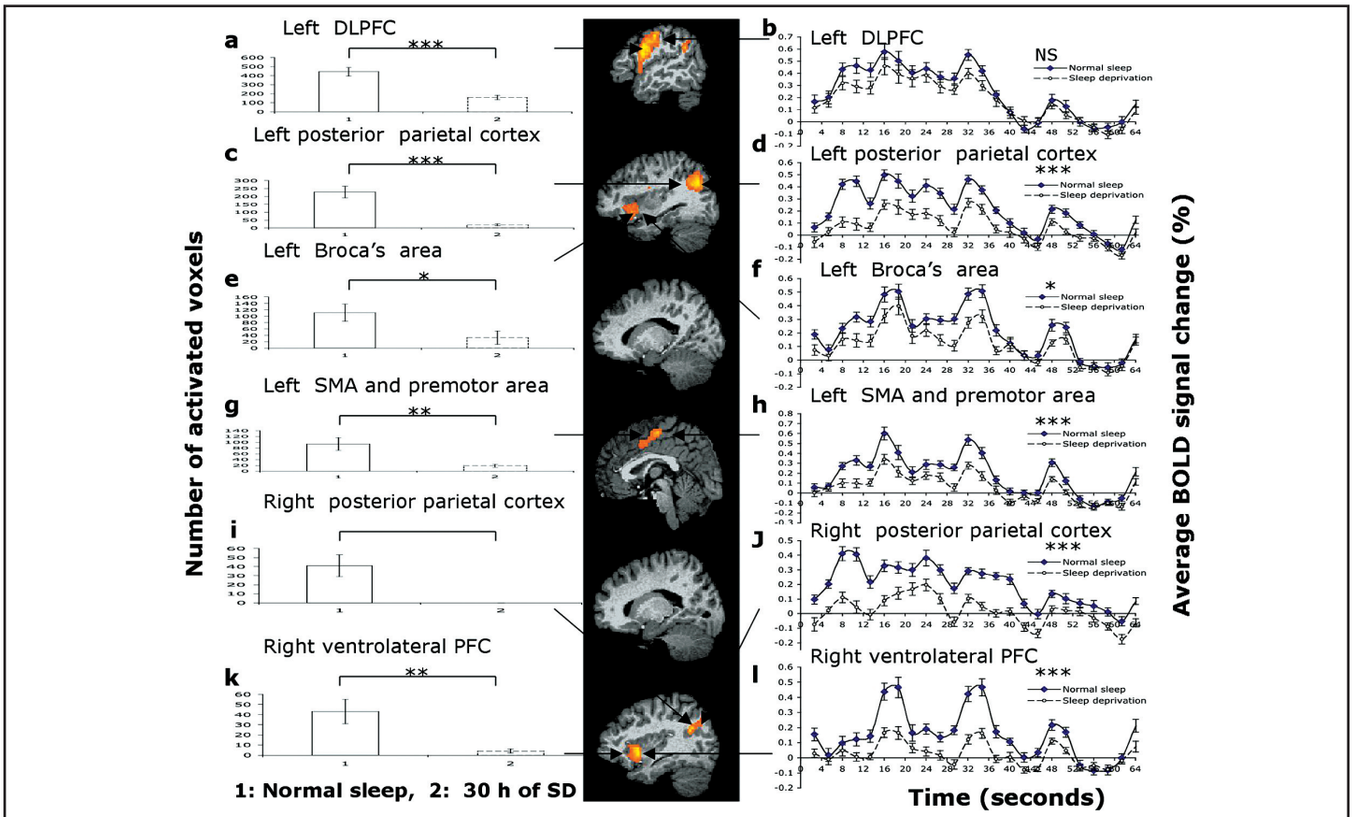

Figure 5—Comparisons of regional activation (normal sleep vs sleep deprivation) (n = 33). a, c, e, g, i, k: Sleep deprivation (SD)-induced significant regional decreases in number of activated voxels. Note no activated voxels in right posterior parietal cortex after SD. b, d, f, h, j, l: Cycle-averaged hemodynamic time curves of regions of interest during Sternberg task show significant decreases in percentage of signal change except the left dorsolateral prefrontal cortex (DLPFC), which does not show significance. The solid line represents the condition of normal sleep, and the dashed line represents the condition of SD. Note that the bilateral parietal cortexes (d) and (j) show similar patterns of time curves, and the left Broca's area (f), supplementary motor area (SMA) and premotor area (PMA) (h), as well as right ventrolateral prefrontal cortex (l), also show similar patterns of time curves. The left DLPFC and parietal regions show relatively sustained patterns of time curves as compared to others. Error bars represent SEM. NS refers to nonsignificant; *P < .05; **P < .005; ***P < .0001.

Table 2—Characterization of Task Difficulty-related Activated and Deactivated Brain Regions Following Normal Sleep and Sleep Deprivation in 30 Men

Brain region	BA	MNI coordinates (mm) X	Y	Z	Cluster size Number of voxels	T value
Normal sleep (within-state analysis)						
Activated (1 letter > control)						
Left dorsolateral PFC	9,45,46	-50	2	22	460	6.72
Left posterior parietal cortex	7,40	-34	-68	30	962	6.02
Left Broca's area	44	-36	22	-16	211	4.75
Left SMA and premotor area	6	-2	-14	60	134	4.54
Right ventrolateral PFC	44	34	14	-2	198	4.53
Right dorsolateral PFC	46	42	-4	22	67	4.51
Right posterior parietal cortex	40	30	-78	22	260	4.99
Deactivated (control > 1 letter)						
None						
Sleep deprivation (within-state analysis)						
Activated (1 letter > control)						
Left dorsolateral PFC	46	-46	10	18	175	4.95
Left posterior parietal cortex	40	-32	-76	14	67	4.52
Deactivated (control > 1 letter)						
None						
Normal sleep > sleep deprivation (1 letter) (between-states analysis)						
Left posterior parietal cortex	40	-46	-56	30	292	4.48
Sleep deprivation > normal sleep (1 letter) (between-states analysis)						
None						
Normal sleep (within-state analysis)						
Activated (3 letters > control)						
Left dorsolateral PFC	9,45,46	-60	-4	38	444	6.22
Left posterior parietal cortex	7,40	-34	-68	38	667	5.07
Left SMA and premotor area	6	-4	-18	58	113	5.42
Right posterior parietal cortex	40	28	-78	28	203	4.92
Left cerebellum		-48	-60	-34	132	4.85
Deactivated (control > 3 letters)						
None						
Sleep deprivation (within-state analysis)						
Activated (3 letters > control)						
Left dorsolateral PFC	46	-58	-4	40	144	5.01
Deactivated (control > SWMT)						
None						
Normal sleep > sleep deprivation (3 letters) (between-states analysis)						
Left dorsolateral PFC	46	-38	-12	40	72	4.28
Left posterior parietal cortex	7,40	-34	-48	58	111	3.43
Left SMA and premotor area	6	-8	-34	66	329	3.87
Right posterior parietal cortex	7,40	28	-78	32	128	3.43
Sleep deprivation > normal sleep (3 letters)(between-states analysis)						
None						

Table 2—*Continued on next page.*

tion times significantly correlated with global activation of PSC ($r = 0.37$, $P < .05$) and significantly correlated with regional activations of the left PPC ($r = 0.52$, $P < .003$) and the right PFC ($r = 0.42$, $P < .05$). That is, slower subjects had more activation, both globally and in these 2 regions. There was no significant correlation between reaction times and global activation. as measured by the NAV that met a standard threshold of statistical significance during the SWMT compared to the control task. The relationship between performance and regional activation was only found during the sleep-deprivation scan. During the screening and rested states, there was no significant correlation between reaction times and global or regional activation.

In terms of task difficulty-related correlational analyses, only the numbers of errors during the 6-letter tasks at the sleep-deprivation state were found to be negatively correlated with global activation ($r = -0.498$, $P < .007$) and with the left PPC ($r = -0.497$, $P < .008$). Thus, less brain activation relates to more errors. No significant performance (reaction times, errors) corre-

Table 2—Continued

Normal sleep (within-state analysis)						
Activated (6 letters > control)						
Left dorsolateral PFC and ventrolateral PFC	9,45,46	-48	4	28	8088	11.82
Left posterior parietal cortex	7, 40	-30	-78	24	3041	10.92
Left SMA and premotor area	6	-6	-4	50	3674	9.24
Right dorsolateral PFC	46	36	40	24	429	5.49
Right posterior parietal cortex	7,40	30	-76	24	1527	7.53
Left Cerebellum		-44	-66	-36	474	6.49
Deactivated (control > 6 letters)						
None						
Sleep deprivation (6 letters)(within-state analysis)						
Activated (6 letters > control)						
Left dorsolateral PFC	9,45,46	-56	-4	34	2007	7.27
Left posterior parietal cortex	7, 40	-34	-60	26	1073	5.97
Left SMA and premotor area	6	-8	-4	50	1722	7.26
Left Broca's area	44	-38	18	6	1004	6.72
Right ventrolateral PFC	44	38	16	-8	490	5.62
Deactivated (control > 6 letters)						
None						
Normal sleep > sleep deprivation (6 letters) (between-states analysis)						
Left dorsolateral PFC	9,45,46	-40	32	24	1426	5.36
Left posterior parietal cortex	7,40	-36	-68	34	2385	5.75
Left Broca's area	44	-52	26	-12	464	5.40
Left SMA and premotor area	6	-4	-2	44	942	5.09
Right ventrolateral PFC	44	34	18	2	122	4.16
Right posterior parietal cortex	7,40	30	-84	26	1163	4.95
Right dorsolateral PFC	9,45,46	48	-2	42	332	4.09
Right premotor area	6	14	-10	72	323	3.99
Left cerebellum		-44	-66	-36	97	3.55
Sleep deprivation > normal sleep (6 letters) (between-states analysis)						
None						

The identical threshold used for cluster analysis was uncorrected $P < .001$ (corrected for multiple comparisons) in the within-state analyses with a spatial extent of $P < .05$ (corrected for multiple comparisons), and an uncorrected $P < .01$ with a spatial extent of $P < .05$ (corrected for multiple comparisons) in the between-states analysis. MNI refers to Montreal Neurological Institute; BA, Brodmann area; SWMT, Sternberg Working Memory Task; PFC, prefrontal cortex; SMA: supplementary motor area. Voxel size = 2 × 2 × 2.

lation with brain activation was found during the 1-letter or 3-letter tasks at the sleep-deprivation state or at the rested state.

DISCUSSION

Only a few functional imaging studies have investigated the effects of sleep deprivation on the human brain during working-memory tasks. Using positron emission tomography during a series of addition/subtraction tasks, Thomas et al[10] found a significant decrease in global cerebral metabolic rate for glucose (CMRglu) and a significant decrease in both absolute and relative regional cerebral metabolic rate for glucose in the PFC and PPC after 24 hours of sleep deprivation. Utilizing fMRI during arithmetic tasks, Drummond et al[8] found a marked decrease in blood oxygenation level-dependent signal after 35 hours of sleep deprivation in regions involved in an arithmetic working-memory task, such as the bilateral PFC, parietal cortexes, and PMA. These studies provided imaging evidence that PFC is particularly vulnerable to sleep deprivation and supported Horne's PFC hypothesis.[3] Recently, Habeck et al[11] explored 48 hours of sleep-deprivation effects using event-related fMRI during a delayed-match-to-sample task, and they found decreased activation in parietal, temporal, and occipital lobes but noted increased activation in the cerebellum, basal ganglia, thalamus, and anterior cingulate gyrus. Although the task they used was adapted from the Sternberg task and thus resembled in some part the current study, their particu-

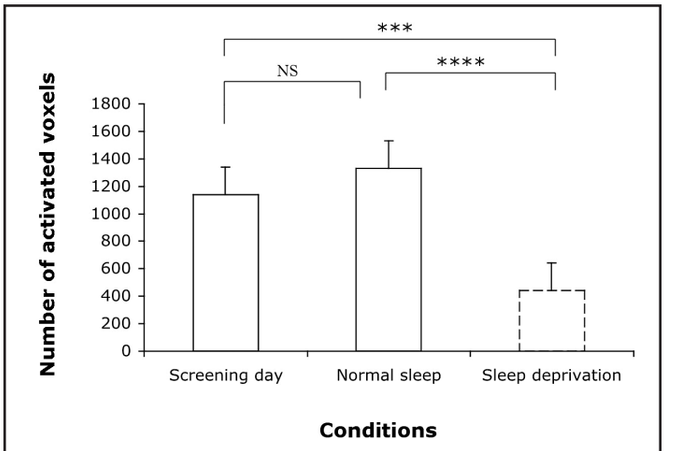

Figure 6—Testing repeatability of global activation across 3 scans (n = 22). No significance between the screening day and normal sleep. Error bars represent SEM based on repeated-measures analysis of variance. NS: nonsignificant; ***P < .005; ****P < .0001.

lar design and interest focused on the probe phase of the task. They compared the probe phase of the Sternberg task to the intertrial intervals, which was not designed as a control task. The differences found during their Sternberg task thus also included nonspecific activation involved in task performance (such as memory scanning, binary decision, response selection and motor output process as stated by the authors) and were not constrained uniquely to working-memory processes. Alternatively, we were interested in understanding sleep-deprivation effects on all the components of verbal working memory with a larger sample. Contrasting the SWMT with a control task to remove potential confounds relating to the task (such as visual, and motor processes), we sought to detect activation changes with sleep deprivation that were unique to verbal working memory. Furthermore, detailed information on quantitative comparisons of the global and regional activations, including patterns of hemodynamic responsive time courses following 30 hours of sleep loss relative to normal sleep, was provided in our present study.

Consistent with previous imaging studies of verbal working memory after a normal night of sleep,[17-19] performing the SWMT resulted in significant activation in the bilateral PFC, PPC, and the left SMA (as well as the right SMA, left PMA, and bilateral anterior cingulate gyri). Confirming our prestudy hypothesis, after 30 hours of sleep loss, subjects had significantly reduced global and regional activation while performing this task. In line with the prior studies in the literature, following 30 hours of sleep deprivation, there was less activation in the left dorsolateral PFC during the SWMT. This supports the view that the PFC is vulnerable to sleep deprivation, derived in part from previous positron emission tomography and fMRI studies[8,10] using arithmetic working-memory tasks. While the current study coincides with the prior findings, however, in terms of activation, the left dorsolateral PFC was the least-affected region in terms of the relative decrease from the rested state to the sleep-deprivation state (for example, this region had no significant change in PSC following sleep deprivation). Thus the PFC was affected following sleep deprivation, but it was only marginally changed compared to other regions. Interestingly and in contrast to the prior studies, the bilateral PPC showed the greatest decrease following sleep deprivation of all activated regions. These parietal regions likely mediate the short-term storage and retrieval of phonologically coded verbal material.[13,14,22] Similar patterns of time-course activation in the bilateral PPC agree with the identical role that they play in the process of verbal working memory. Although a control task was used to minimize the nonspecific verbal components, it is still possible that the parietal activations relate to visual and spatial working-memory processing; for example, subjects may use the visual or spatial strategy to store some target arrays. That is, the parietal activation might simply reflect some adjunct processing of information from the Sternberg task, with the PFC being the real locus of control. It is possible that the PFC was impaired to a marked degree and that the parietal area simply followed suit.

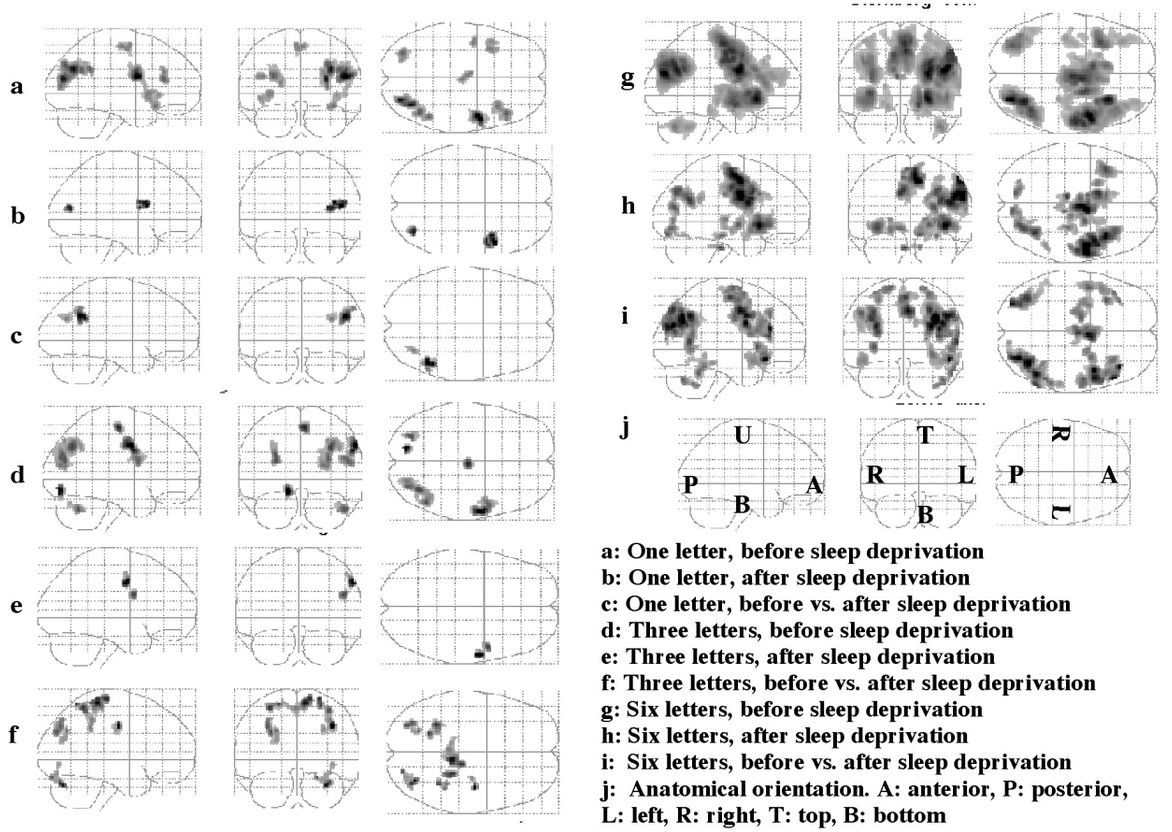

Figure 7—Task-difficulty related brain activation following normal sleep and sleep deprivation in 3-D projections of SPM glass-brains. a, d, g represent brain activation induced by 1-letter, 3-letter, and 6-letter tasks following normal sleep; b, e, h display brain activation induced by 1-letter, 3-letter, and 6-letter tasks following sleep deprivation. Maps were thresholded at $P < .001$ with a spatial extent $P < .05$ (corrected for multiple comparisons). c, f, i show direct comparisons between the rested state and the sleep-deprived state during 1-letter, 3-letter, and 6-letter tasks. Maps were thresholded at $P < .01$ with a spatial extent $P < .05$ (corrected for multiple comparisons). j indicates anatomical orientation of 3-D projections at sagittal, coronal, and axial views.

In addition to the decreased activation in these areas, significant reduction in cortical functional response to the SWMT was also noted in anterior speech regions (Broca's area, SMA and PMA), suggesting that sleep deprivation interferes with verbal rehearsal in this subsystem that is specialized for the rehearsal of phonologic information.[13,14] Interestingly, these areas showed similar patterns of time-course activation, implying that they perform the same or similar functions during the verbal working-memory task. The current study found that sleep deprivation produced marked decreases in cortical response to SWMT in the proposed verbal working-memory areas, both globally and regionally, expressed as both NAV and PSC. These results suggest that not only the PFC, but also other verbal working memory-involved areas, especially the parietal cortex, are vulnerable to sleep deprivation. The data thus confirm Horne's hypothesis of PFC vulnerability to sleep deprivation.[3] However the PFC was less vulnerable than other regions, particularly the PPC and anterior speech areas. Using both the NAV and PSC, the present study provides neuroimaging evidence that the parietal regions, bilaterally, are more vulnerable to sleep deprivation than are other regions involved in verbal working memory, including the dorsolateral PFC. This differs from the results of previous arithmetic working-memory studies,[8,10] which found that the PFC is more vulnerable to sleep deprivation than the parietal cortex. Moreover, following sleep deprivation, the decreased cortical response we found with this working-memory task stands in contrast to the increased brain responses following sleep deprivation to verbal learning[7] and to grammatical transformation.[15] These discrepancies support the cognitive task-specific hypothesis that the functional effects of sleep deprivation in the brain may vary, at least in part, with specific tasks.[7-11,15,32]

Although the parietal mechanism of sleep deprivation remains unknown, the functional cortical response to sleep deprivation may be working-memory task specific rather than brain-region specific.[32] Additionally, the left hemisphere has been demonstrated to be dominant for verbal processes.[13,14,17-19,22] However, in the present study, the right hemisphere also showed significant decreases in parietal cortex and ventrolateral PFC activation following sleep deprivation, implying that the left and right hemispheres are both involved in verbal working memory and are both vulnerable to sleep deprivation. It is also possible that the left inferior frontal gyrus responded to the encoding of the stimuli while the right hemisphere responded to the recognition component of the Sternberg task.

Another possibility, however, needs to be considered regarding the task-specific sleep-deprivation effects. Following sleep deprivation, although we found significant decrease in global PSC during the Sternberg tasks, we also found significant decrease in global PSC during the control tasks, implying the possibility that sleep-deprivation effects may not be task specific. The control tasks used in our study involved very simple visual and motor, as well as binary-choice processes. The decreased change in global brain activation seen in the control tasks supports the notion that simple monotonous tasks are sensitive to sleep deprivation. Because of the susceptibility of the Sternberg and the control tasks to sleep deprivation, it is conceivable that sleep deprivation produced decreased brain activation in both tasks. To our knowledge, this is the first time the hemodynamic responsive time curves in functional imaging studies following sleep deprivation have been presented. The significance of decreased activation in the control tasks needs to be further investigated in the future when interpreting functional imaging data. For example, when sleep deprivation induces various (decreased, increased, or nonsignificant) changes in brain activation during various cognitive tasks (relative to the control tasks), will the brain activation change during control tasks and, if so, how?

A potential limitation of the current study is the lack of a counterbalanced design. Several research groups to date have used an uncounterbalanced design with normal healthy subjects[5,6,10,11] commonly using a group without sleep deprivation to control the potential task-practice effects. While we did not use a control group without sleep deprivation, a repeated-measures design was instead employed to clarify potential scan-order effects. On the basis of recent reports that the Sternberg task is sensitive to practice effects[33] and that extensive practice diminishes practice-related changes in brain activation during working memory,[34] all the subjects had practiced well (10 times) with the STWM and reached an asymptotic performance level prior to the first screening scanning sessions (see Figure1b). A repeated ANOVA revealed that subjects reached an asymptotic performance level after 4 times of practice. This finding is accordant with a recent study during a Sternberg task conducted by Habeck et al,[11] in which nonsignificant practice effects were found after subjects went through a training run of 7 blocks, tested using a control group without sleep deprivation. Pilot studies also showed that practice effects resolved in another verbal task, while preserving the differential difficulty of sentence levels, after 4 administrations.[15] Furthermore, both the performance and brain activation between the screening state and rested state were investigated, and no significant difference in performance was found (see results for reaction times and error comparisons). No significant difference was found in global brain activation (see Figure 6) and regional activation (no significant difference between the map comparison of the 2 states). The nonsignificant difference in brain activation and performance between the screening and rested states indicates that 1 more practice at the rested state (compared to the screening state) did not produce significant practice-related confounds; in other words, the practice effects were not significant confounds between these 2 states, although the scan order was not counterbalanced. Given that all subjects had practiced the task to an asymptotic level prior to the screening scanning session and that there was no significant changes in both the brain activation and performance between the screening and the rested states, it seems to be unlikely that 1 more practice at the sleep-deprivation state (relative to the rested state) would result in a significant practice-related change in brain activation at such well-practiced conditions; that is, there would likely be no significant practice effects between the rested state and a third scan, the sleep-deprivation state (for each subject, there was only 1 more practice at the rested state than at the screening state and there was only one more practice at the sleep-deprivation state than at the restated state due to the scan order in which subjects performed the SWMT while they were being scanned within the MRI scanner). Because we failed to directly differentiate the potential practice effects between the rested and sleep-deprivation states, we acknowledge that the potential confounds may still exist, although they might be relatively small.

In agreement with previous behavioral and functional imaging studies that sleep deprivation in humans causes performance decrements,[3,5,8,11] sleep deprivation caused significant declines in

performance and increases in sleepiness while subjects performed the SWMT within the scanner. The exact relationship between the decrease in cerebral activation and the change in behavioral performance is unknown. Regarding this link, uniquely, on the scan after sleep deprivation, reaction times significantly correlated with global activation as well as with specific activation in the parietal and PFC. Also, only at the sleep-deprivation state did the number of errors during the 6-letter task negatively correlate with the left PFC and PPC activation. These results suggest that many brain regions in addition to the prefrontal cortex, and including the parietal cortex, show changes in activation following sleep loss. Further studies with counterbalanced designs are needed to explore this interesting and important area.

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