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Altered brain response to verbal learning following sleep deprivation

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The effects of sleep deprivation on the neural substrates of cognition are poorly understood. Here we used functional magnetic resonance imaging to measure the effects of 35 hours of sleep deprivation on cerebral activation during verbal learning in normal young volunteers. On the basis of a previous hypothesis[1], we predicted that the prefrontal cortex (PFC) would be less responsive to cognitive demands following sleep deprivation. Contrary to our expectations, however, the PFC was more responsive after one night of sleep deprivation than after normal sleep. Increased subjective sleepiness in sleep-deprived subjects correlated significantly with activation of the PFC. The temporal lobe was activated after normal sleep but not after sleep deprivation; in contrast, the parietal lobes were not activated after normal sleep but were activated after sleep deprivation. Although sleep deprivation significantly impaired free recall compared with the rested state, better free recall in sleep-deprived subjects was associated with greater parietal lobe activation. These findings show that there are dynamic, compensatory changes in cerebral activation during verbal learning after sleep deprivation and implicate the PFC and parietal lobes in this compensation.

Being deprived of sleep for one night impairs performance on many cognitive tasks[2–6]. Verbal learning is a critical cognitive function whose susceptibility to the detrimental effects of sleep deprivation (SD) has been particularly well replicated[7–9]. Decreases in specific cognitive functions after SD may be associated with impairments in the cerebral systems that form the neural substrates of these functions[1]. In particular, SD has been reported to impair performance on cognitive tasks, including verbal learning tasks, that are putatively dependent upon PFC involvement[4–6]. These observations led us to propose that some cerebral systems, particularly the PFC, would be less activated by cognitive tasks in sleep-deprived than in rested subjects. To test this proposal directly requires the use of noninvasive techniques that measure localized cerebral function. To our knowledge, only four reports using functional brain-imaging methods have described the effects of SD on cognitive performance[10–13]. Although none of these studies investigated the effects of SD on verbal learning, two of them reported reduced cerebral metabolic rate in the PFC following SD[10,12].

We measured localized cerebral activation using the blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) method during performance on a verbal learning task in 13 normal young volunteers after a normal night of sleep (the rested state) and after 34.7 ± 1.2 hours without sleep (the SD state). For each state, the cognitive task alternated between a baseline condition (determining whether a list of nouns was in upper or lower case) and an experimental condition (memorizing a list of nouns)[14]. During task performance, BOLD functional images were acquired at each of 20 sagittally oriented slices covering the whole brain Using high-resolution anatomical images, we identified regions of significant activation during each state separately and regions that were significantly more activated during one state than the other. Significant activation was determined using a cluster threshold method to protect against type I errors[15].

Subjects performed significantly less well on free recall when they were sleep-deprived (4.7 ± 4 words after normal sleep versus 2.8 ± 2 words after SD, $P<0.05$), but showed no significant change in recognition memory (discriminability index, $d' = 2.5 \pm 1$ versus 2.0 ± 1, $P>0.05$). Subjective levels of sleepiness on the Stanford sleepiness scale (SSS)[16] increased (2.3 ± 0.8 versus 4.4 ± 1.4, $P < 0.001$) and levels of concentration decreased (this and subsequent subjective measures used a 5-point Lickert scale; 4.5 ± 0.7 versus 3.5 ± 1.1, $P<0.007$). Subjective estimates of effort (4.0 ± 1.2

Table 1 Regions of significant brain activation following normal sleep and sleep-deprived nights

	Normal sleep			Sleep deprivation	
Brain regions	Talairach Coordinates	Brodmann Areas	Brain regions	Talairach Coordinates	Brodmann Areas
L. MFG	33L, 58A, 6S	10*	L. SFG	18L, 30A, 45S	8
L. MFG/IFG	47L, 24A, 11S	8,9/45,47†‡	L. MFG/OFG	29L, 54A, 4S	10*§
L. OFG	12L, 54A, 14I	10§	L. MFG	52L, 20A, 31S	8/9/46†
L. AC	4L, 31A, 13S	32, 24	L. IFG	53L, 24A, 1I	47‡
L. AC	5L, 21A, 10I	32, 35	L. AC	10L, 24A, 37S	32
L. PMA & SMA	15L, 11A, 61S	6	R. MOG	25R, 92P, 13S	18, 19
	41L, 5A, 25S	6			
	47L, 3A, 42S	6			
	25L, 18A, 53S	6			
L. TP	34L, 4A, 18I	38			
L. MTG	56L, 22P, 5I	21			
L. SOG	43L, 72P, 37S	19			

Each entry represents a significant cluster of activation. Clusters that physically overlap between nights are denoted with identical symbols (*, †, ‡ or §). Premotor area (PMA) and supplementary motor area (SMA) activation after the normal night of sleep included four discrete clusters. The anterior cingulate (AC) gyrus activation after the SD night was slightly dorsal (superior) to that observed after the normal night of sleep. SFG, MFG, IFG and OFG: superior, middle, inferior and orbital frontal gyri, respectively; MTG: middle temporal gyrus; SOG and MOG: superior and middle occipital gyri, respectively. L, left hemisphere; R, right hemisphere. The magnitude of activation of every pixel of each cluster was significant at a minimum *t*-value of 2.18, degrees of freedom 12.

versus 4.1 ± 1.0) and of task difficulty (1.2 ± 0.4 versus 1.6 ± 0.8) did not change significantly between the rested and sleep-deprived states.

In the rested state, the left PFC, premotor area and temporal lobe were activated during the verbal learning task (Table 1). Contrary to our hypothesis, discrete regions of the PFC were more activated during verbal learning following SD than following normal sleep. The temporal lobes were significantly more activated during the rested state than during the SD state (Fig. 1a). Additionally, the bilateral parietal lobes (Fig. 1b) and two additional frontal lobe regions (left middle frontal gyrus and right inferior frontal gyrus) were more activated after SD than in the rested condition. These regions are involved in tasks with high working memory or cognitive loads[17–20]and also include the areas hypothesized to be the site of the short-term memory store[20,21].

Simple repetition of the verbal learning task might alter BOLD response through practice effects or habituation. To determine whether brain areas that showed altered BOLD response following sleep deprivation also showed altered BOLD response induced by repetition of the verbal learning task, we performed a paired *t*-test comparing the first and second nights of activation. There was no overlap between areas showing sleep deprivation effects and areas showing repetition effects.

To explore further the relationships among cerebral activation, SD and cognitive performance, we regressed cerebral activation during the SD night onto participants' subjective levels of sleepiness and onto their free recall performances. Increased sleepiness was significantly correlated with activation in two bilateral PFC regions (left inferior frontal gyrus at BA47 and right superior/middle frontal gyri at BA10) and one in the right cerebellum. Furthermore, better free recall after SD was associated with greater activation in three regions: within the bilateral parietal lobes and one each in the right temporal gyrus and the left supplementary motor area. Subjective ratings of effort and of task difficulty obtained during SD did not significantly correlate with activation of the brain sites that were more responsive during SD.

Several authors have argued that some aspect of cerebral dysfunction following SD impairs cognitive performance compared with the rested state[1,3,5–6]. This study partially supports this argument (there is loss of left temporal lobe activation after SD), and is consistent with a preliminary report suggesting that SD alters the overall pattern of cerebral activation during task performance[13]. Our data also indicate that the brain may be able to compensate for the effects of SD while maintaining at least partially intact performance. Following SD, performance is often initially intact and then declines with increasing time-on-task, suggesting that individuals can initially compensate for the effects of SD. Here, SD led to increased BOLD signals during verbal learning in bilateral working memory regions of both the frontal and parietal lobes. Thus, we propose that these regions may represent the neurophysiological substrate of the initial compensation for SD. Such a compensatory mechanism has been proposed for cognitive performance during other types of stressor[22]. Given the relatively short duration of our task, we may have only measured that period of time when the brain is able to compensate for SD and maintain relatively intact performance. It is possible that had we used a longer task, we might have observed a smaller compensatory brain response.

Following SD, activation within PFC was significantly correlated with sleepiness, whereas activation within the parietal lobes was related to preservation of near-normal verbal learning. The significant relationship between increased prefrontal cortical response to verbal learning and increased sleepiness is of particular interest for two reasons. First, during non-rapid eye movement (nonREM) sleep, increased delta frequency in the electroencephalogram (the frequency associated with deep slow-wave sleep) occurs earlier in the prefrontal cortex than in the rest of the cortex, especially after SD[23]. Second, levels of adenosine in the basal forebrain rise monotonically with increasing time spent awake and might be responsible for the induction and maintenance of nonREM sleep[24]. One might speculate, then, that the increased prefrontal response to verbal learning after SD reflects compensation for the direct or indirect effects of adenosine, or of other endogenous sleep-promoting substances in the basal forebrain. Under this hypothesis, the increased prefrontal cortex response to verbal learning after SD would represent compensation for the increased homeostatic drive for sleep. The increased parietal activation seen after SD, on the other hand, may underlie partially successful behavioural adaptation to SD during verbal learning. The compensatory response of the parietal lobe to SD might be restricted to cognitive functions that do not activate the parietal lobes during the rested state. For example, serial arithmetic activates the parietal lobes when subjects are rested, and SD impairs serial arithmetic and is associated with a diminished parietal BOLD response to arithmetic[25].

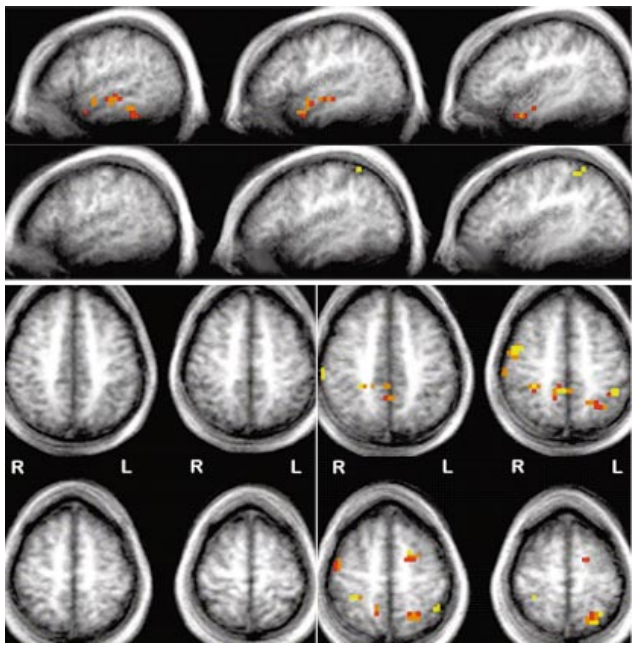

Figure 1 Within-subject significant *t*-tests for mean differences in activation between rested and SD nights. Red, smallest differences; yellow, largest; $n = 13$. **a,** Both panels show left hemisphere sagittal slices from lateral to medial at 51, 47 and 43 mm from midline (left to right). Top: areas showing increased activation after normal sleep. Areas not shown include the anteria angulate, left premotor area and medial and left cerebellum. Bottom: areas showing increased activation after SD. **b,** Both panels show axial slices above the inter-commissural line (clockwise from upper left: 43, 48, 58 and 53mm). Left: no significant activation in any area in these slices after normal sleep. Right: increased activation after SD. Additional areas include left middle frontal gyrus, right inferior frontal gyrus, right inferior temporal gyrus and superior occipital gyrus.

Although changes in effort might affect neural activation in brain regions associated with working memory, increased effort is unlikely to explain the increased PFC and parietal response to verbal learning following SD. Neither subjective levels of effort nor perceived task difficulty changed with SD or correlated significantly with localized brain activation following SD.

Behavioural compensation for SD was not complete, and some of the changes in cerebral activation that followed SD may have contributed to poorer recall performance. In particular, the reduced response of the left temporal lobe to verbal learning following SD might have been associated with lowered recall scores. Decreased temporal lobe activity is associated with poor verbal learning in

pathological conditions, such as Alzheimer's disease, where reduced perfusion of the temporal lobe predicts the rate of decline of verbal memory[26].

In summary, the neural mechanisms mediating cognitive performance after SD are complex and dynamic, and differ, in part, from those employed in the rested state. The effects of SD on cognitive performance and functional cortical activation illustrate the brain's plasticity and, at times, its attempts to compensate for the failure of normal neural systems during the execution of specific tasks. In the case of verbal learning, haemodynamic responses increased following SD in both the frontal lobes (perhaps responding to or reflecting sleepiness) and in the parietal lobes (perhaps assisting with the achievement of near-normal free recall) despite an apparent loss of temporal lobe involvement. In contrast, in the case of serial subtraction[25], haemodynamic responses were significantly reduced after SD in the frontal and parietal lobes as performance faltered. These observations indicate that the effects of SD on cognitive performance and related patterns of cerebral activation may depend in part on task-specific demands. □

Methods

Subjects and behavioural conditions

Thirteen normal healthy subjects (mean age 27.2 years, range 21–35 years; mean education 16.5 years, range 14–18 years) participated after providing written informed consent. All subjects were screened using a medical and psychiatric history, physical and laboratory examinations and an overnight sleep laboratory evaluation to establish that they had relatively normal sleep patterns. During SD, subjects were monitored in a hospital from 22:00 until the time of the scan, around 16:30–18:00 the next day, and were not allowed stimulants of any kind. The order of the rested and SD conditions was counterbalanced. Subjects performed four separate cognitive tasks while undergoing fMRI scans in both the rested and SD states. The study of serial subtraction has been reported elsewhere[25]. The present study concerns verbal learning, as described below.

The behavioural trials alternated between four experimental and five baseline blocks, starting and ending with a baseline block (each block, 40 s; total trial, 360 s). Five words were presented during each block; subjects were told to not memorize the baseline words, but to determine whether they were in all upper-case or all lower-case letters. Subjects were instructed to actively memorize the experimental words for later testing. Free recall and recognition were tested outside the scanner about 10 min after the end of the functional scans. We analysed free recall data using a paired-samples *t*-test, and recognition memory performance using the discriminability index *d′*. After completion of the memory tests, subjects rated, on a series of 5-point Lickert scales, their subjective levels of concentration, effort and the difficulty of the task. In addition, participants rated subjective sleepiness before and after the scanner procedures[16].

fMRI procedures and data analysis

All images were acquired with a 1.5T GE Signa scanner equipped with an inserted three-axis balanced torque head gradient coil designed for rapid switching[27]. Whole-brain manual shimming further enhanced the signal-to-noise ratio. Functional images consisted of 90 repetitions of echo planar images (TR, 4,000 ms; TE, 40 ms; FOV, 24 cm; image matrix, 64×64; in-plane size, 3.75 mm^2; slice thickness, 6 mm; no interslice gap) acquired continuously in an interleaved fashion. For anatomical images, we collected 128 1.5-mm contiguous images using the magnetization prepared rapid acquisition gradient echo (MPRAGE) protocol (256×256 matrix; FOV, 24 cm; flip angle, 10^o).

All analyses were conducted using Analysis of Functional NeuroImages (AFNI) software[28]. After motion correction, the functional time-course data from each voxel were correlated with a series of 13 reference functions—one seed reference function representing the alternating time course of the behavioural trial and the same reference function shifted in 1-s increments six times both forwards and backwards in time[14]. AFNI then used only the reference function that produced the highest correlation with the time-course data. Shifting the reference function takes into account the delays in onset of the haemodynamic response and in the acquisition of the first and final slices[29]. These correlations were performed within-subject before combining data during group analyses. Before performing group analyses, all individual data sets were transformed into standard atlas[30] and spatially smoothed with a Gaussian kernel equal to 3.75 mm full-width-half-maximum. Within-night analyses consisted of performing one-sample *t*-tests on each voxel with a null hypothesis that the contrast between the experimental and control blocks averaged across subjects equaled zero. Between-night analyses consisted of performing paired samples *t*-tests with a null hypothesis that the average signal contrast between the experimental and control blocks on each night was equal. All *t*-tests examined the image intensity derived from the correlation of the time-course data with the reference functions as the dependent variable and used the variance in image intensity across subjects to test for significant BOLD response in each individual voxel. Hypotheses were related to areas significantly more activated by the experimental task than the baseline task; therefore, only positive activation was examined. The regression analysis performed on the SD data used the increase in SSS scores between the normal sleep scan and the SD scan (SD – normal sleep) to control for basal levels of sleepiness. The change in free recall performance from the normal to the SD night was used to estimate the degree to which SD affected performance (normal sleep – SD: larger values represent greater impairment). Both positive parameter estimates (areas associated with greater performance decrements) and negative parameter estimates (areas associated with more intact performance) were examined for this variable.

The cluster thresholding method to control for type I error associated with multiple statistical tests was as described[15]. Based on 10,000 Monte Carlo simulations, we defined a significant activation as a cluster of at least six contiguous voxels, each individually activated at an *a priori* alpha level < 0.025 (1-sided). This resulted in a final per-voxel *P*-value of 0.0005 (1-sided).

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