Dissociation of cortical regions modulated by both working memory load and sleep deprivation and by sleep deprivation alone

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Working memory is an important mental capacity that is compromised following sleep deprivation (SD). To understand how working memory load interacts with state to influence brain activation in load-sensitive regions, and the extent to which SD-related changes are common across different loads, we used fMRI to study twelve healthy subjects following 24 h of SD using a verbal *n*-back task with three load levels. Performance decline was observed by way of reduced accuracy and slower response times following SD. The left prefrontal region and thalamus showed load dependent activity modulation that interacted with state. The right parietal and anterior medial frontal regions showed load dependent changes in activity as well as an effect of state. The anterior cingulate and occipital regions showed activation that displayed state effects that were independent of working memory load. These findings represent a step toward identifying how different brain regions exhibit varying vulnerability to the deleterious effects of SD on working memory.
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Introduction

Sleep deprivation (SD) is an important facet of modern life that is inescapable for many. A recent US National Sleep Foundation study found that 40% or fewer adults get at least 7 h of sleep on weekday nights. SD impairs performance in a number of cognitive tasks, but the extent to which it does so varies according to the cognitive domain tested. Tasks that engage the frontal lobes are well studied and among those that are vulnerable to SD (Jones and Harrison, 2001). The vulnerability of the frontal lobes to SD is further supported by the finding that predominantly frontal EEG changes occur after SD (Cajochen et al., 1999). Given that the frontal lobes are crucial for working memory (WM), it is not surprising that SD has an adverse effect on working memory.

Working memory is important to study because it underpins many higher cognitive processes. However, working memory itself is not a unitary entity and may be studied in terms of stimulus modality (verbal, non-verbal), temporally-defined components (encoding, delay period and probe-cued retrieval), process-defined components (maintenance vs. manipulation), and item load. Understanding how SD affects these elements is pivotal to helping us identify those that are particularly vulnerable to SD and should be avoided when accurate performance is critical. Furthermore, understanding the neural substrate underlying these vulnerable elements might provide us with ways to temporarily ameliorate the effects of SD using behavioral or pharmacologic approaches. In order to fulfill this goal, it is necessary to systematically evaluate the effect of SD on each element. Using this approach is supported by the divergent results of neuroimaging studies of task performance following SD. These studies illustrate that activation patterns following SD are exquisitely sensitive to task (Bell-McGinty et al., 2004; Drummond and Brown, 2001; Drummond et al., 2004).

Existing studies of WM during SD show that in some instances, decrements in performance may be correlated with reduced frontal activation (Drummond et al., 1999; Thomas et al., 2000) whereas in other instances performance is somewhat indifferent to changes in frontal activation (Habeck et al., 2004). We recently demonstrated that in accordance to prior behavioral studies, following SD, greater task complexity may paradoxically result in better preserved performance (Chee and Choo, 2004). This may in turn be associated with greater prefrontal and thalamic activation. In these experiments, the more complex task required additional manipulation of items held in WM while the less complex task required maintenance of items in WM.

In studies that did not involve SD, increasing task complexity by increasing task load generally results in increasing prefrontal

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activation (Braver et al., 1997; Callicott et al., 1999). At higher task loads, however, prefrontal activation has been found to increase monotonically (Braver et al., 1997) or peak and then decline in an inverted-U shape manner (Callicott et al., 1999). A decline in PFC activation at high WM loads has been interpreted as supporting evidence for a capacity constrained WM system (Callicott et al., 1999).

At present, it remains unclear how increasing difficulty in the context of a WM task interacts with SD to influence brain activation. In a recent experiment utilizing one-, three-, or six-letter arrays in a delayed-match-to-sample task (Habeck et al., 2004), the same level of reduced PFC activation was observed across all three load levels. In contrast, another study that involved WM manipulation and engagement of semantic processing, increasing task difficulty resulted in increased activation in several brain regions following SD including the left frontal and inferior parietal regions (Drummond et al., 2004). Given these conflicting results, we sought to clarify the manner in which task load interacts with state to influence brain activation as working memory is engaged following SD.

While the functional anatomy of the frontal lobes is the focus of many studies, the extent to which SD modulates cortical activation in other regions is also of interest. Some of these changes appear to generalize across different tasks and may be conceived as task independent but state dependent effects. For example, reduction in parieto-occipital activation with SD has been observed across several different tasks (Bell-McGinty et al., 2004; Chee and Choo, 2004; Drummond et al., 1999, 2001; Habeck et al., 2004). Activity in the anterior cingulate has been found to increase following SD in a variety of tasks (Drummond and Brown, 2001; Drummond et al., 2004; Habeck et al., 2004). Reduced deactivation in the anterior medial frontal regions has been reported in two working memory tasks following SD (Chee and Choo, 2004). Although hinted at by Habeck et al. (2004), the generalizability of these findings merits further evaluation.

In light of past findings, we set ourselves two goals in the present study: to find a set of load-sensitive regions where load and state interact to modulate brain activation, and to find regions that show state-dependent effects across all loads. To accomplish these objectives, we scanned subjects using fMRI during rested wakefulness (RW) and following SD in a counterbalanced block design experiment. WM load was varied using a verbal *n*-back paradigm. We expected to find (WM) load-sensitive regions in the frontal and parietal lobes during RW (Braver et al., 1997; Callicott et al., 1999). Further, we hypothesized that in the presence of a capacity constrained WM system the detrimental effect of SD would be more evident at higher loads and that this would manifest as a reduction in activation during SD relative to RW at the same WM load. Lastly, we predicted that regions whose activation is influenced by SD but not by task load would lie in the occipital, anterior cingulate, and anterior medial frontal regions and that our results might extend those obtained recently (Habeck et al., 2004).

Methods

Subjects

14 right-handed, neurologically normal subjects (9 males; mean age 21.8 ± 0.8 years) were recruited from local tertiary institutions for the experiments. Data from 2 subjects were discarded because they did not achieve the required performance accuracy of 80% (as a result of lapses occurring during scanning after being sleep deprived). Informed consent was obtained and subjects were paid for their participation. Prior to participation, subjects kept a sleep diary for 1 week and only subjects with regular sleeping habits (sleeping no later than 1 am, waking no later than 9 am, and no habitual daytime napping) were studied. None of the subjects were on medication and recreational drug use was excluded.

Experimental protocol

Subjects were scanned twice, once during rested wakefulness (RW) and once following sleep deprivation (SD). The two scanning sessions were conducted 1 week apart and the order of scanning was counterbalanced across subjects to reduce the potential influence of practice, learning, and order effects on brain activation. Subjects abstained from smoking, caffeine, and other stimulants for 24 h prior to being scanned. While undergoing SD, subjects were monitored in the laboratory from 2100 h onwards. They were allowed to engage in non-strenuous activities such as watching videos and conversing. Every hour throughout the study night and under supervision, subjects rated their sleep propensity using the Epworth sleepiness scale (ESS) and performed a simple reaction time task (SRT). The SRT required that subjects respond by pressing the appropriate key, depending on whether they saw a left- or right-pointing arrow. Arrows appeared at random (1.0 s to 5.0 s) after the start of each trial. 180 trials were executed during each testing session. Scanning took place after 24.4 ± 0.3 h of wakefulness. To guard against the confounding effect of circadian rhythm on cognitive performance between the two scan sessions, both the RW and SD scans were commenced at the same time of day, i.e., between 0730 and 0930 h (for a commentary on this issue, see Drummond et al., 2004).

Experimental task

The experimental stimuli consisted of pseudorandom sequences of letter consonants that were presented in either uppercase or lowercase. Subjects had to indicate if the probe letter matched the stimulus presented '*n*-back' ago using a hand-held response box (Fig. 1). For the 0-back task, subjects indicated whether the present stimulus matched a pre-specified letter: 'X'. In all four conditions [i.e., 0-back (0B), 1-back (1B), 2-back (2B), 3-back (3B)], targets were presented in 33% of trials.

Each experimental run consisted of four control (0-back) blocks that alternated with each of the three task blocks (1B, 2B, 3B) in counterbalanced order. Prior to each task block, a cue indicating the task to be performed appeared for 2900 ms. In each block, ten trials were performed where each letter appeared for 500 ms before being replaced by a fixation cross for 2400 ms. Each experiment block lasted 31.9 s, inclusive of the cue. Prior to each scanning session, subjects practiced the task until a minimum level of performance (>80% accuracy on all load levels) was achieved. Eight experimental runs of the task were performed in each scanning session. The order of task block presentation was counterbalanced across test sessions and across subjects.

MRI scanning procedure

Stimuli were projected onto a screen using a LCD projector and viewed through a rear-view mirror. Subjects responded by
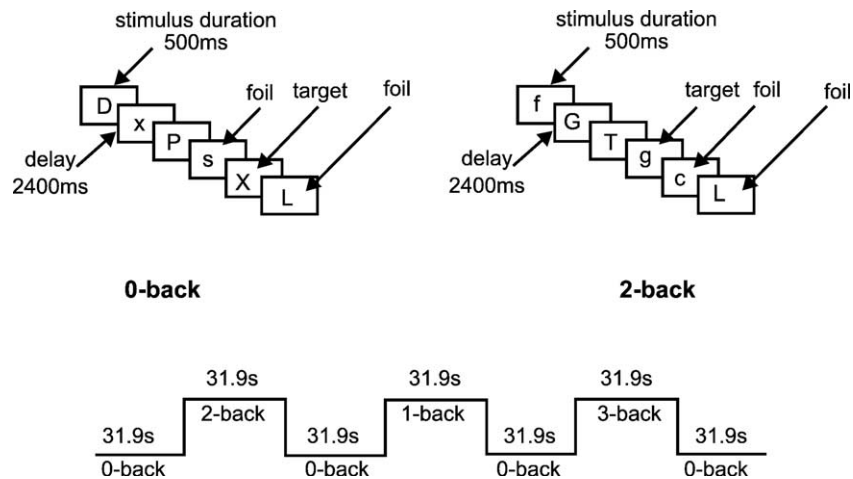
Fig. 1. Task stimuli and timing parameters. The 0-back condition served as the control condition. The 1-back, 2-back, and 3-back conditions were counterbalanced across runs and across subjects.

pressing buttons on a hand-held response box with the right hand. A bite-bar was used to reduce head-motion. Images were acquired on a 3T Allegra MRI system (Siemens, Erlangen, Germany). A gradient-echo EPI sequence was used with TR = 3000 ms, FOV = 192 × 192 mm, and 64 × 64 mm pixel matrix. 32 oblique axial slices with thickness 3 mm (0.3 mm gap) approximately parallel to the AC–PC line were acquired. High-resolution coplanar T2 weighted anatomical images were also obtained. A further high-resolution anatomical reference image was acquired using a T1 weighted 3D-MPRAGE sequence for the purpose of image display in Talairach space.

Image analysis

Motion-correction was performed in-scanner using PACE (Siemens, Erlangen, Germany). Functional images were processed with Brain Voyager 2000 v.4.9 (Brain Innovation, Maastricht, Holland). Mean intensity normalization was performed to obtain the same average intensity for each slice across scans. Between slice timing differences due to slice acquisition order were adjusted using sinc interpolation. Gaussian filtering was applied in the spatial domain using a smoothing kernel of 8 mm FWHM for group-level activation maps. Intra-session image alignment to correct for motion across runs was performed using the first image of the functional run that was acquired immediately prior to a coplanar T2 weighted image, as the reference image. The T2 images were used to register the functional data set to the volunteers' own 3D image. The resulting aligned data set was then transformed into Talairach space. The group level anatomical image was an arithmetical average of individuals' structural images.

Functional analysis was performed using a GLM with six predictors-of-interest ($1B_{RW}$, $2B_{RW}$, $3B_{RW}$, $1B_{SD}$, $2B_{SD}$, $3B_{SD}$), and a confound predictor for each run. To identify load-responsive regions during RW, the contrast ($2B_{RW} > 1B_{RW}$) was evaluated using a random effects analysis. We chose this analysis strategy because prior studies have shown sigmoid (Cohen et al., 1997), quadratic (Callicott et al., 1999), and linear (Rypma et al., 1999) patterns of BOLD signal modulation in response to working memory load. The chosen approach detects regions showing increasing activation with load but does not

make assumptions concerning the specific pattern of how load modulates activation (at higher load levels) allowing for linear and quadratic responses to be detected without bias. A threshold of $P < 0.001$ (uncorrected) was used in the evaluation of this contrast (Braver et al., 2001; Cabeza et al., 2002) and the cluster threshold was set at 6 contiguous voxels (each functional voxel being 3 mm × 3 mm × 3 mm).

Voxels fulfilling this threshold during RW were used to define regions of interest (ROI) that were evaluated in the SD condition for a hypothesis driven analysis of activation. This analysis sought to detect an interaction between state and load in regions showing sensitivity to load during RW. It will detect regions showing greater or lesser modulation of activation as a function of load following SD. We chose to anchor our analysis on regions known to be modulated by load during rested wakefulness because there is a wealth of literature characterizing these regions and their role in working memory. Suprathreshold voxels in these ROIs within a search volume of size 15 mm × 15 mm × 15 mm centered on the activation peak were analyzed. Parameter estimates of signal change from each individual were obtained from these ROIs and compared between states using post hoc, paired t tests for each load level. As parameter estimates obtained from each individual represents an independent sample in the second level tests, a significance level of $P < 0.05$ was chosen to evaluate each comparison. As this analysis strategy will not detect regions that show modulation of working memory load following SD but not during RW, we performed a second analysis

Table 1
Behavioral responses obtained during rested wakefulness (RW) and following sleep deprivation (SD)

	1-Back	2-Back	3-Back
Accuracy			
RW	0.988 (0.013)	0.968 (0.050)	0.943 (0.052)
SD	0.941 (0.050)	0.927 (0.074)	0.911 (0.086)
RT (ms)			
RW	552 (149)	588 (162)	617 (233)
SD	668 (182)	746 (271)	718 (245)

Response time (RT) is reported in milliseconds (ms). Standard deviation appears in parentheses.

Table 2
Regions sensitive to working memory load during RW

Region	BA	x	y	z	t
L inferior frontal gyrus	44	−46	10	24	6.1
R middle frontal gyrus	9	35	31	34	5.6
L anterior cingulate gyrus	32	−2	22	44	4.6
L inferior parietal lobule	39	−32	−50	35	8.3
R inferior parietal lobule	39	31	−63	36	5.9
R precuneus	7	4	−68	48	7.0
L thalamus	–	−16	−20	18	5.7
L anterior medial frontal cortex[a]	10	−7	49	26	5.2

Results were based on the contrast ($2B_{RW} > 1B_{RW}$) (random effects analysis, threshold $P < 0.001$, uncorrected).
[a] Deactivation.

using voxel-by-voxel ANOVA to uncover these regions (see below).

To identify regions whose activation was state-dependent and irrespective of load, i.e., consistent across all load levels, we identified suprathreshold voxels that fulfilled a conjunction of three contrasts [($1B_{SD} > 1B_{RW}$), ($2B_{SD} > 2B_{RW}$), and ($3B_{SD} > 3B_{RW}$)]. We chose this analysis because an ANOVA approach where the main effect of state is probed would identify regions where activity differs at some but not all levels of working memory load. The method is very conservative in that the regions identified through this process surpassed the statistical threshold of $P < 0.001$ in all the contrasts of interest. As such, the t values cited henceforth indicate the comparison showing the contrast with the smallest signal difference (Nichols et al., 2005).

As a secondary analysis, we used a voxel-based ANOVA approach to identify regions showing a load by state interaction and regions showing a main effect of state. To identify these regions, ANOVA was performed on a voxel-by-voxel basis using a random effects analysis. This routine was performed with an in-house plug-in routine developed by VV. The statistical and cluster

thresholds used to select voxels were identical to those used in the hypothesis driven approach ($P < 0.001$ uncorrected, random effects analysis).

Results

Behavioral data

Out of scanner ESS and SRT data from 4 subjects and in-scanner n-back task performance, data from 1 subject were lost due to technical errors. Subjects reported increased sleep propensity following SD, as reflected by the increase in ESS [$t(7) = 7.2$, $P < 0.001$]. Response times on the SRT were slower [$t(7) = 3.0$, $P < 0.05$] and more variable [$t(7) = 2.4$, $P < 0.05$] following SD. Performance on the n-back task declined following SD (Table 1). A repeated measures ANOVA for accuracy showed a main effect of state [$F(1,11) = 6.6$, $P < 0.05$] and a trend toward a main effect of load [$F(2,11) = 3.3$, $P < 0.08$] with no significant interaction. There was a significant difference in performance when accuracy in 3B was compared to 1B during rested wakefulness (RW) [$t(11) = 2.6$, $P < 0.05$] and also when 3B was compared to 2B in RW [$t(11) = 2.8$, $P < 0.05$]. For response times, a significant main effect of state [$F(1,11) = 14.9$, $P < 0.005$] was present. There was no significant effect of load or interaction. Run order did not have a significant effect on RT during RW or following SD in the baseline condition (0 back) (see Supplementary Fig. 1).

Imaging results: load-sensitive regions

Load-sensitive regions during wakefulness were identified in the left inferior frontal gyrus (BA 44) extending into the left middle frontal gyrus (BA 9), right middle frontal gyrus (BA 9), left anterior medial frontal cortex (BA 10), left anterior cingulate
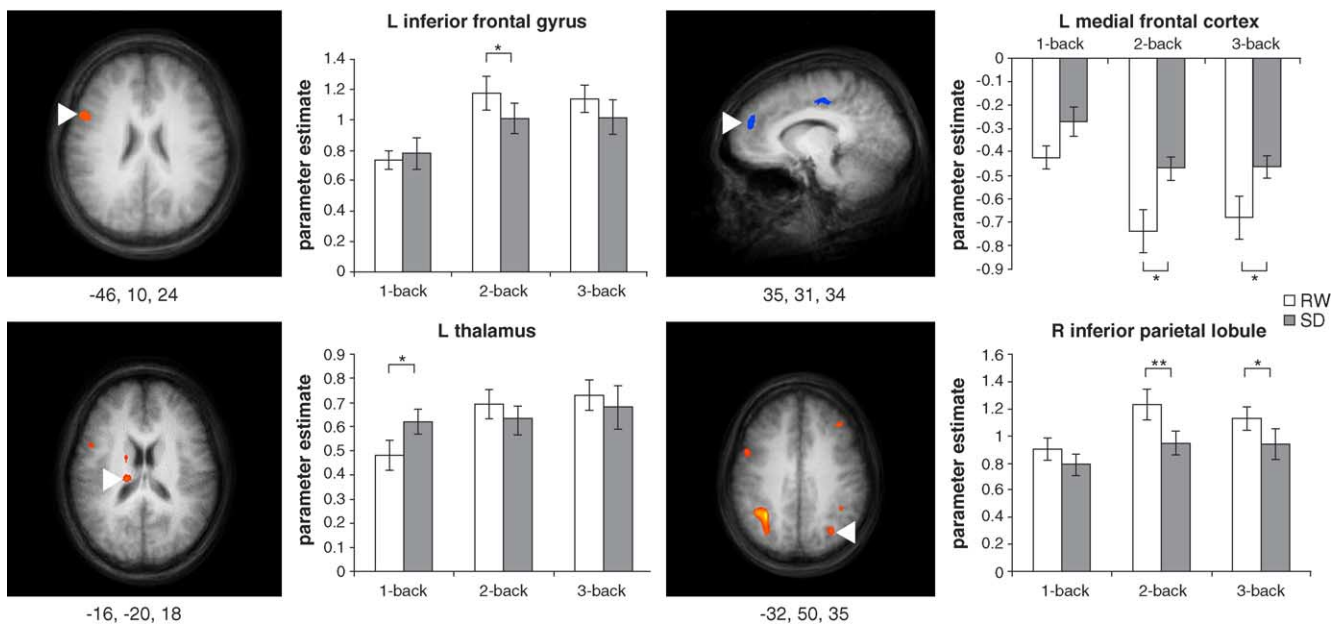

Fig. 2. Activation in regions sensitive to working memory load during RW and following SD. Each region was selected using the contrast ($2B_{RW} > 1B_{RW}$) (random effects analysis, threshold $P < 0.001$ uncorrected). Corresponding parameter estimates of activation in these ROI across load and state are depicted in the bar graphs alongside each figure. Error bars denote standard error. Significant differences between states are denoted as follows: *$P < 0.05$, **$P < 0.005$.

Table 3
Regions where activity was consistently modulated by state

Region	BA	x	y	z	t value
SD-related increase					
L anterior cingulate cortex	32	−7	31	36	3.4
SD-related decrease					
L middle occipital gyrus	18	−29	−83	21	4.0
R middle occipital gyrus	18	19	−78	15	3.7

Results were based on a conjunction analysis of state-related differences across all load conditions (random effects analysis, threshold $P < 0.001$, uncorrected).

gyrus (BA 32), bilateral inferior parietal regions (BA 39), right precuneus (BA 7), and left thalamus (Table 2). Almost all of these load sensitive areas showed activation that increased with load in the same manner during RW and SD. In these load-sensitive regions, a capacity constrained pattern of activation was seen

whereby there was either no significant difference or a decline in activation from 2B to 3B.

Effect of SD on load-sensitive regions: hypothesis driven approach

In load-sensitive regions identified from the RW state, task related BOLD signal change showed significant load by state interaction in the left dorsolateral prefrontal cortex at the superior end of the inferior frontal gyrus [$F_{(2,10)} = 3.9$, $P < 0.05$] and left thalamus [$F_{(2,10)} = 4.2$, $P < 0.05$] (Fig. 2). Reduced activation in the left inferior frontal gyrus following SD was observed at 2B [$t(11) = 2.3$, $P < 0.05$]. Although not statistically significant, there was a trend toward a reduced activation after SD at 3B.

Within the anterior medial frontal region, a main effect of state [$F_{(1,11)} = 6.9$, $P < 0.02$] was observed. There was no significant load by state interaction. The state effect was manifested as reduced deactivation following SD (Fig. 2). A main effect of state
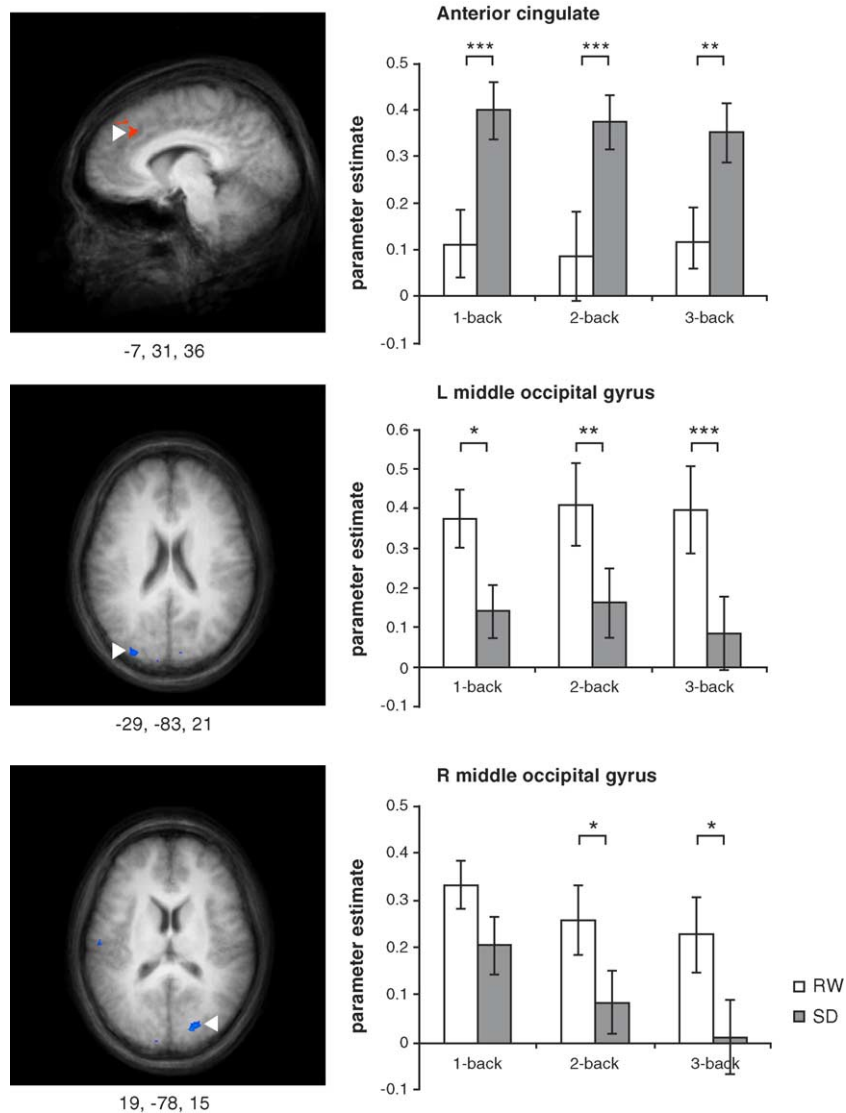

Fig. 3. Regions showing consistent effects of state across different load levels using the conjunction analysis outlined in the text (random effects analysis, threshold $P < 0.001$ uncorrected). Corresponding parameter estimates of activation in these ROIs across load and state are depicted in the bar graphs alongside each figure. Error bars denote standard error. Significant differences between states are denoted as follows: *$P < 0.05$, **$P < 0.005$, ***$P < 0.001$.

Table 4
Results of the voxel-based ANOVA showing Talairach coordinates of activation peaks of regions showing a main effect of state and those showing load by state interaction

Region	BA	x	y	z	F value
State effect					
SD-related increase					
L thalamus	–	−8	−19	4	9.1
SD-related decrease					
L middle occipital gyrus	19	−29	−80	17	18.4
R middle occipital gyrus	19	29	−80	16	19.4
R lingual gyrus	17/18	18	−77	19	19.6
R lingual gyrus	17/18	19	−77	20	20.0
L middle temporal gyrus	21/22	−60	−18	−1	15.9
Load by state interaction					
L inferior frontal gyrus	44	−34	9	25	14.0
R middle frontal gyrus	10	24	51	7	6.5
R insula	13	30	26	1	7.4

The F values are higher than those reported in the hypothesis driven approach because the statistics here are reported for the peak voxels.

was also observed in the right inferior parietal region [$F(1,11) = 7.1$, $P < 0.05$] whereby there was reduced activation following SD. In all other load-sensitive regions, there was neither a significant effect of state and nor any interaction.

State-dependent, load-independent regions

Following SD, the left anterior cingulate gyrus (BA 32) showed increased activation to a similar extent across all load levels; neither significant effects of load nor interaction were observed (Table 3; Fig. 3). Similarly, a reduction in BOLD response to the same extent across different loads following SD was seen in the left middle occipital gyrus (BA 18) (Table 3; Fig. 3). Note that the right middle occipital gyrus showed reduction in BOLD response following SD with a significant effect of load [$F(2,10) = 7.1$, $P < 0.02$].

Secondary analysis using voxel-based ANOVA approach

The secondary analysis using the voxel-based ANOVA approach concurred with the hypothesis driven approach in showing a similar pattern of load by state interaction in the left inferior frontal gyrus (BA 44) [$F(2,10) = 14.0$, $P < 0.002$] (Table 4; Fig. 4). This region showed higher activation with increasing load in both states but relatively lower activation following SD compared to RW in the 2 or 3 back load conditions. These regions are those that the parametric design might be expected to detect as they show activity variations according to what one might expect of regions involved in working memory.

In contrast, regions showing load by state interaction but whose activation was not increased consistently with increasing load were the right middle frontal gyrus (BA 10), right insula (BA 13), and bilateral lingual gyri (BA 17). Activation in these regions is not further discussed, as these regions have not been documented to play a role in verbal working memory.

The effects of state in the anterior cingulate (BA 32), bilateral inferior parietal lobules (BA 39), and both occipital lobes (BA 18) were more extensive (Fig. 4) as might be expected. This is because ANOVA approach is less strict than multiple conjunctions in defining state dependent regions.

Discussion

The results of the present study show that it is possible to dissociate brain regions whose activity is modulated by both working memory load and SD from regions where SD exerts an effect independent of item load. With respect to working memory load sensitive regions, the left prefrontal region showed an interaction between load and state whereas bilateral occipital and anterior medial frontal regions showed main effects of state without interaction. The anterior cingulate and occipital regions showed state modulated activation that was consistently seen at all three working memory loads.

Effects of sleep deprivation on cortical activation in working memory load-sensitive regions

During RW, we identified a bilateral frontal–parietal network of WM load-sensitive regions similar to that found in previous experiments that utilized a verbal n-back paradigm (Braver et al., 1997; Callicott et al., 1999; Honey et al., 2000). Within these regions, left prefrontal activation increased from 1B to 2B but either did not increase or showed a trend toward decrement at 3B in keeping with the suggestion that in some implementations of the n-back task, activation does not rise monotonically as a function of increasing load even under optimal conditions, supporting the notion that working memory has limited capacity and that this capacity limitation can be demonstrated with functional brain imaging (Callicott et al., 1999).

Following SD, there was less left prefrontal activation in 2B compared to during RW, with a corresponding reduction in task performance in SD. This is interesting in light of two recent investigations evaluating the effects of task difficulty in a parametric fashion involving at least three levels of task difficulty (Drummond et al., 2004; Habeck et al., 2004).

In the study involving logical reasoning, there was increased left prefrontal and parietal activation corresponding to maintained performance after SD. It was concluded that increasing task difficulty facilitated cerebral compensatory responses (Drummond et al., 2004). In concurrence with this finding, we recently showed that the additional requirement of having to manipulate items in working memory (as opposed to merely maintaining them) resulted in better-preserved performance. This was accompanied by disproportionately greater left prefrontal and thalamic activation following SD (Chee and Choo, 2004).

Two aspects of the logical reasoning task might account for the differences between that study and the present findings. Firstly, the cognitive processes required for the logical reasoning task differ from those engaged in the n-back task in that access to semantic and syntactic processes is required in addition to visuo-spatial working memory processes (Drummond et al., 2003). Secondly, we cannot exclude circadian factors as a cause of differences in results. We scanned subjects in the morning, following 24 h of SD, when subjects were close to the nadir of the circadian cycle (Dawson and Reid, 1997; Falleti et al., 2003) and where performance decline is maximal (see Supplementary Fig. 2). In the experiments performed by Drummond et al. (2004), subjects were scanned at 35 h of SD, close to the 'wake maintenance zone' where sleep is least likely to occur and performance is expected to be less severely affected (Drummond and Brown, 2001).

In the second parametric load-varying experiment, a delayed-match-to-sample task was employed. Following SD, decreases in
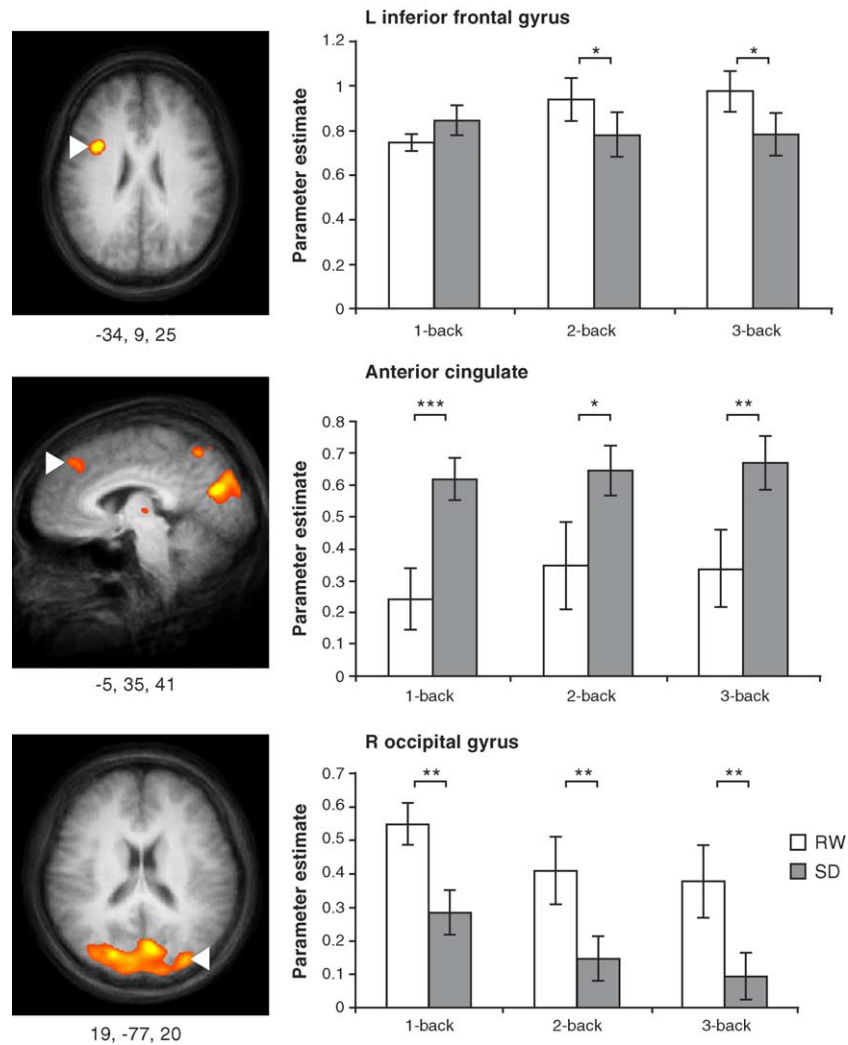
Fig 4. Results of the secondary analysis using voxel level ANOVA (random effects analysis, threshold $P < 0.001$ uncorrected). A load by state interaction was identified in the left prefrontal region (top panel); a main effect of state was observed in the anterior cingulate (middle panel) and occipital (bottom panel) regions. Corresponding parameter estimates of activation in these ROI across load and state are depicted in the bar graphs alongside each figure. Error bars denote standard error. Significant differences between states are denoted as follows: *$P < 0.05$, **$P < 0.005$, ***$P < 0.001$.

brain activation in a consistent set of regions that included bilateral parietal, occipital, and mid-temporal areas occurred across all load levels (Habeck et al., 2004). This experiment entailed SD of 48 h (evaluating volunteers at the nadir of the circadian cycle) and showed a decline in performance. There were main effects of load and state but there was no interaction.

In general, increased activation corresponds to relatively preserved performance whereas decreased activation correlates with performance decline. However, the lack of a consistent load by state relationship across different tasks suggests that while task difficulty or complexity may sometimes positively influence performance (Chee and Choo, 2004; Drummond et al., 2004; Portas et al., 1998), this is not invariably so (Bell-McGinty et al., 2004; Habeck et al., 2004). It is also significant to note that no single area in which activation is increased following SD consistently tracks performance maintenance. Candidate regions include the left prefrontal region (Chee and Choo, 2004; Drummond et al., 2004; Portas et al., 1998), the inferior parietal regions (Drummond et al., 2000, 2004), and the thalamus (Chee and Choo, 2004; Portas et al., 1998).

We found that cortical activation peaks at different load levels for different regions in the two states; whereas PFC and parietal activation peaked at 2B, thalamic activation did so at 1B. The Ordinal Trend Canonical Variates Analysis (OrTCVA) method used by Habeck (Bell-McGinty et al., 2004; Habeck et al., 2004) provides a measure to quantify increase or reduction in the covariance pattern across states but does not inform us if activation peaks at different load levels in various regions of interest. Our approach complements the OrTCVA method by directly evaluating hypothesis driven regions of interest.

Task related deactivation of anterior midline frontal regions

Deactivation, as we use the term, refers to a reduction of BOLD signal during task performance (1B, 2B, 3B) relative to the control task (0B) (Gusnard and Raichle, 2001). This occurred in the medial frontal regions during goal-directed processing and may represent the suppression of 'self referent' behavior (Frith and Frith, 1999; Gusnard et al., 2001). The magnitude of this deactivation has been reported to increase with increasing task

difficulty (McKiernan et al., 2003) and the effect of load on deactivation in the present experiment is in keeping with this observation. It is important to point out that unless the control task is considered, reduced deactivation may be construed as increased activation. This might in fact have been observed in previous studies (Drummond and Brown, 2001; Drummond et al., 2001).

The state-related reduction in deactivation may be interpreted as contributing to performance impairment. In previous work that also examined working memory following SD, we found that reduced deactivation in this region following SD correlated with slower individual performance (Chee and Choo, 2004). With healthy elderly subjects whose performance accuracy was poorer than in young controls, the magnitude of midline frontal deactivation was also reduced (Lustig et al., 2003).

State dependent effects common to all load levels

Following SD, reduced activation in the occipital regions extending to the inferior parietal region was recorded at all load levels. A similar reduction of activation following SD in the occipital region has been observed in prior studies (Bell-McGinty et al., 2004; Chee and Choo, 2004; Drummond et al., 1999; Habeck et al., 2004) that used different working memory tasks and tested volunteers across different load levels. This result suggests that the reduction in parieto-occipital activation may be a task and load independent (consistent across loads) effect of SD. It has been suggested that SD may affect the processing of visual object properties at the perceptual and attentional level (Bell-McGinty et al., 2004).

Increased anterior cingulate (ACC) activation following SD is consistent with findings obtained from studying divided attention and working memory using a delayed match to sample task (Drummond et al., 2001; Habeck et al., 2004). SD presents a situation where performance is impaired and where increased errors are likely. Activation in the ACC is associated with conditions under which errors are increased (Ullsperger and von Cramon, 2001); this region has also been implicated in detecting situations where errors are likely (Carter et al., 1998). The increased ACC activation following SD may therefore relate to these processes.

Conclusion

The varied results obtained from imaging studies on following SD suggest that studying the neural correlates of memory in this setting is more complex than originally envisaged. We have attempted to study working memory function in SD from the perspective of varying load and have shown regions that are sensitive to load, state, and the interaction of load and state. Our results support a systematic approach to testing each related dimension (task component processes, performance, duration of SD, and circadian influences) prior to establishing generalizable principles regarding the functional anatomy of working memory following SD.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2004.11.029.

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