

# Functional imaging of working memory following normal sleep and after 24 and 35 h of sleep deprivation: Correlations of fronto-parietal activation with performance

Michael W.L. Chee,<sup>a,\*</sup> Lisa Y.M. Chuah,<sup>a</sup> Vinod Venkatraman,<sup>a</sup> Wai Yen Chan,<sup>a</sup> Pierre Philip,<sup>b</sup> and David F. Dinges<sup>c</sup>

<sup>a</sup>Cognitive Neuroscience Laboratory, SingHealth Research Laboratories, 7 Hospital Drive, #01-11, Singapore 169611, Singapore

<sup>b</sup>Sleep Clinic, Bordeaux-Pellegrin Hospital, France

<sup>c</sup>University of Pennsylvania School of Medicine, Department of Psychiatry, Philadelphia, PA 19104, USA

Received 19 October 2005; revised 29 November 2005; accepted 2 December 2005

Available online 19 January 2006

**Working memory was evaluated after normal sleep, and at 24 and 35 h of sleep deprivation (SD) in 26 healthy young adults to examine the neural correlates of inter-individual differences in performance. The extent of performance decline was not significantly different between the two SD test periods although there was greater variability in performance at SD35. In both SD sessions, there was reduced task-related activation (relative to normal sleep) in both superior parietal regions and the left thalamus. Activation of the left parietal and left frontal regions after normal sleep was negatively correlated with performance accuracy decline from normal sleep to SD24 thus differentiating persons who maintained working memory performance following SD from those who were vulnerable to its effects.**

© 2005 Elsevier Inc. All rights reserved.

**Keywords:** Working memory; Parietal lobe; Prefrontal cortex; Sleep deprivation; fMRI; PVT

## Introduction

Sleep deprivation (SD) is associated with performance decline in a wide range of cognitive tasks (Pilcher and Huffcutt, 1996; Durmer and Dinges, 2005). Recent evidence indicates substantial inter-individual differences in this performance decline, and that this differential vulnerability to cognitive impairment following SD may be trait-like (Van Dongen et al., 2004). The cognitive vulnerability to sleep loss clusters on three distinct neurobehavioral dimensions: (1) self-evaluation of sleepiness, fatigue and mood; (2) cognitive processing capability (e.g., working memory) and (3) behavioral alertness as measured by sustained attention performance (Van Dongen et al., 2004).

The first goal of the present study was to examine how inter-individual differences in brain activation correspond to working memory task performance in the context of sleep deprivation. We were specifically interested in whether functional imaging can serve as a marker for predicting performance decline following SD. We evaluated working memory as this cognitive domain engages fronto-parietal networks (Curtis and D'Esposito, 2003) whose activation is influenced by sleep deprivation (Bell-McGinty et al., 2004; Chee and Choo, 2004; Chee et al., 2004; Habeck et al., 2004; Mu et al., 2005a,b). Of particular interest, Mu et al. (2005a), using a Sternberg-type working memory task, reported that individuals who were vulnerable to working memory impairment following SD were those with higher global brain activation prior to SD (also see Caldwell et al., 2005) and less reduction in global activation following SD, suggesting the viability of using imaging to predict resistance to cognitive decline following SD.

In the present study, we evaluated working memory decline in SD, using a different set of working memory tasks that were presented in a counterbalanced manner. We reasoned that if brain imaging is to be useful in predicting differential vulnerability to SD, results should replicate across different tests evaluating the same cognitive domain. Such reproducibility has been evaluated in behavioral testing (Frey et al., 2004; Van Dongen et al., 2004; Frey et al., 2005), but not with functional imaging.

Concurrent with the goal of identifying the neural correlates of resistance or vulnerability to working memory performance decline following SD, we also investigated the effect of scanning at different time points following SD on brain activation. Previous imaging studies have involved 24 (Thomas et al., 2000; Chee and Choo, 2004; Choo et al., 2005), 30 (Mu et al., 2005b), 35 (Drummond et al., 2000; Doran et al., 2001; Drummond and Brown, 2001; Drummond et al., 2004) and 48 h (Bell-McGinty et al., 2004; Habeck et al., 2004) of total SD but, to date, imaging has been performed only at a single time point following SD. The non-uniformity of imaging results obtained from different studies (Table 1) has been attributed

\* Corresponding author. Fax: +65 62246326.

E-mail address: mchee@pacific.net.sg (M.W.L. Chee).

Available online on ScienceDirect (www.sciencedirect.com).

Table 1  
Summary of brain imaging studies involving sleep deprivation

Reference	Domain tested	SD duration	<i>N</i>	Frontal activity in SD relative to RW	Parietal activity in SD relative to RW	Other areas
Chee and Choo (2004)	Working memory (maintenance and manipulation)	24	14	Increased with task complexity	Decreased	Increase in thalamus
Choo et al. (2005)	Working memory ( <i>n</i> -back)	24	12	Decreased with task load	Decreased	
Habeck et al. (2004)	Working memory (Sternberg-type delayed-match-to-sample [DMS] task with three levels of load)	48	18	No change	Decreased	
Mu et al. (2005b)	Working memory (Sternberg-type verbal working memory task)	30	33	Decreased	Decreased	Decrease in thalamus
Bell-McGinty et al. (2004)	Working memory (DMS task with visual shapes)	48	19	No change	Decrease in the precuneus	
Drummond et al. (2004)	Baddeley's logical reasoning task (WM component)	35	16	Increased	Increased	
Drummond et al. (2004)	Serial subtraction	35	13	Decreased	Decreased	Decrease in the premotor area
Thomas et al. (2000) (*PET study)	Serial addition/subtraction	24	17	Decreased	Decreased	Decrease in the thalamus

to task differences (Drummond and Brown, 2001). However, it is also possible that scanning at different time points (SD24 and SD35) could have contributed to the difference in imaging results.

Cognitive performance in the context of SD is known to be modulated by the interaction of two effects: an endogenous, cyclically varying circadian effect as well as an homeostatic effect related to the increasing maintenance of wakefulness (Van Dongen and Dinges, 2005). Depending on how the two effects interact, performance after 35 h of wakefulness, while impaired relative to baseline, can stay the same (Van Dongen and Dinges, 2005) or improve (Doran et al., 2001) relative to 24 h of SD. Replicating SD effects at two different circadian phases increases confidence that the brain changes are due to the elevation of homeostatic sleep drive.

In the present study, we evaluated each volunteer after rested wakefulness as well as after 24 and 35 h of SD, the two most commonly used test times in imaging studies, to determine the comparability of studies performed at SD24 and SD35. A null result in a study recruiting a large number of volunteers would facilitate the interpretation of data emerging from different laboratories that are constrained to perform imaging at particular times for operational reasons. Otherwise, investigators in this field might have to agree on a standardized time to perform such studies.

## Methods

### Volunteer characteristics

Of an original cohort of 41 recruits, 28 healthy, right-handed adults aged between 19–25 years (14 men) completed this study, and of these, 26 volunteers' data were fully analyzed. They were recruited through advertisements placed in university halls of residence. Ethical approval for this study was obtained from the Singapore General Hospital IRB and volunteers were reimbursed for the completion of the experimental protocol. To qualify for recruitment, volunteers had to declare that they:

1. Sleep on the average of 6.5–9 h at night;
2. Have regular sleeping hours whereby they sleep no later than 1 am and rise before 9 am each day;

3. Have no history of excessive daytime sleepiness or insomnia;
4. Be free from psychiatric illness, obstructive sleep apnea, narcolepsy or periodic leg movements as ascertained by questionnaire;
5. Have no history of recreational drug use or excessive alcohol consumption;
6. Have no history of psychoactive drug use for 3 months prior to the study.

### Experimental protocol

Volunteers were asked to present themselves to the laboratory 1 week prior to the first scanning session. In this session, they were introduced to, and practiced on, one complete 'run' of the in-scanner working memory task. They also completed the Raven's Advanced Progressive Matrices (Raven et al., 1998) to evaluate their non-verbal IQ as this has been known to affect the extent of brain activation in working memory tests (Gray et al., 2003). The volunteers were also asked to wear a wrist actigraph for the week prior to their first scan as well as for the entire duration of the study. Volunteers whose wrist actigraphy suggested non-compliance to rules were not studied further. Thirteen volunteers out of an original 41 were rejected on this account.

All participants were scanned three times, once during rested wakefulness (RW) and twice during the sleep deprivation (SD) session (Fig. 1). Each RW scan session took place between 0900 and 1030 h. Scanning following SD took place after approximately 22–24 h of wakefulness, commencing between 0600 h and 0800 h and again after 34–35 h of wakefulness, commencing between 1800 h and 1900 h. The two sessions were conducted at least 1 week apart and the order of these sessions was counterbalanced across subjects. All participants were asked to abstain from smoking, caffeine and other stimulants for 24 h prior to being scanned. Prior to each scanning session, volunteers were practiced on at least one complete 'run' of the working memory task, using stimuli that were not repeated during the actual test.

For the SD session, subjects were monitored in the laboratory from 1900 h of the first night to 1900 h the following evening. Two subjects were scanned per test night. They were allowed to engage

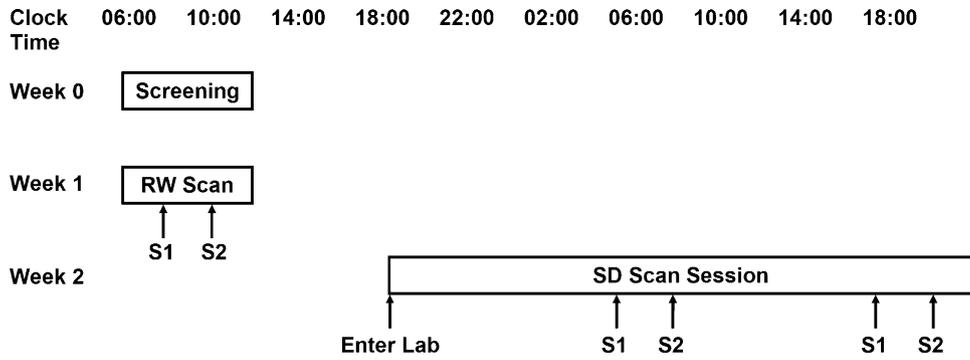


Fig. 1. Study design schematic showing the three phases of the study and the times of day volunteers (S1 and S2) underwent briefing and brain imaging, respectively. The order of scanning was counterbalanced across subjects.

in non-strenuous activities such as watching videos, reading and conversing. Major meals were taken between 1900 h and 2000 h, 0900 h to 1000 h and 1200 h to 1400 h. Snacks were allowed.

As mentioned previously, three neurobehavioral domains have been demonstrated to independently account for some of the inter-individual variability in vulnerability to impairment from sleep loss (Van Dongen et al., 2004). To evaluate the extent to which these measures were related in the present study cohort, we obtained behavioral measures in the self-evaluation of sleepiness (Karolinska Sleepiness Scale or KSS; Akerstedt and Gillberg, 1990) and sustained attention (using a modified version of the Psychomotor Vigilance Task), in addition to the working memory task.

Every hour throughout the SD session, participants rated their sleep propensity on the KSS. A substantially modified version (M-PVT) of the Psychomotor Vigilance Test (Dorrian et al., 2005), implemented on a Palm handheld device (Philip et al., 2004) was administered every hour for 10 min, but not during the periods when the volunteers were in the scanner. In the M-PVT, volunteers responded to the appearance of a black square that appeared at intervals randomly varying from 2 to 7 s and were required to press a button as quickly as possible to turn the square off. If no response

was given within 1750 ms, a new interval was started. Pressing the key before the square was displayed, or within 100 ms of the appearance of the square (advances), caused the response to be discarded and a warning to be displayed. Each administration consisted of 100 trials.

M-PVT performance was assessed by using two performance metrics derived in line with prior studies (Dinges et al., 1997; Graw et al., 2004): (1) RT difference between slowest (90% percentile) and fastest RT (10th percentile) and (2) number of lapses (RT  $\geq$  500 ms). Data obtained hourly between 2300 and 1900 h for RT differences and lapses were each averaged to yield two summary scores, one for RT difference and one for lapses.

*In-scanner experimental tasks*

Three working memory tasks were used (Fig. 2). The LTR task evaluated maintenance and was adapted from previous work on verbal working memory (Reuter-Lorenz et al., 2000). Four different uppercase letters were presented for 0.5 s followed by a delay period of 3.0 s during which a fixation cross was displayed. A probe letter (in lowercase) was then presented for 1.5 s and this

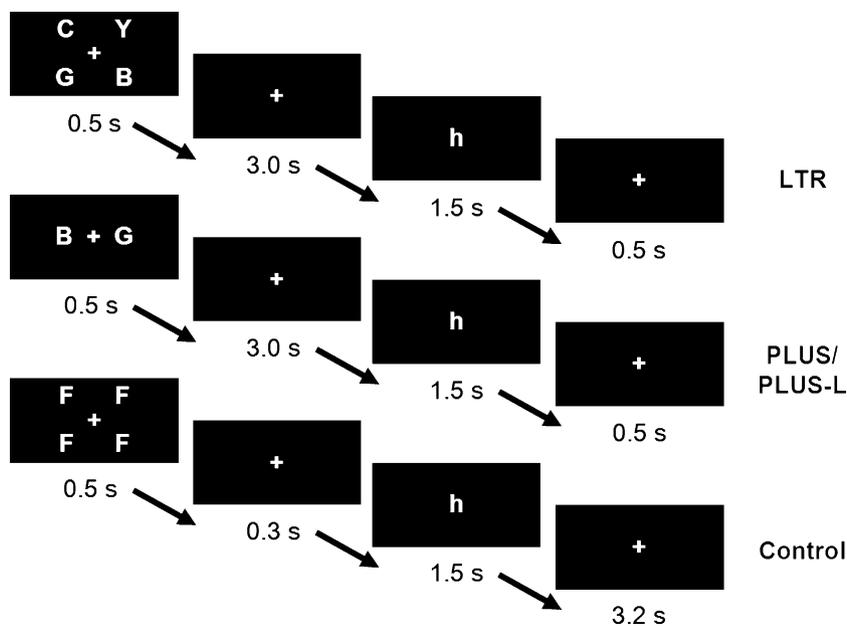


Fig. 2. Exemplars of stimuli used in the in-scanner working memory tasks. The timings indicate the duration of exposure of each stimulus. PLUS and PLUS-L differed in the nature of the probe whereby 50% of the probes in PLUS-L were lures comprising a recently shown letter (e.g., B in the example shown).

was followed by fixation for a further 0.5 s. Subjects signaled a match or a non-match by pressing one of two appropriate response buttons on a MR compatible response system within the duration that the probe letter was present. Half the probes were matches, and the other half, non-matches. Response time information was available on-line.

The PLUS task was designed to engage manipulation of items retained in verbal working memory. Two different letters were presented and subjects were instructed to mentally shift each letter forward alphabetically and to keep in mind the results. For example, if 'B' and 'J' were presented, subjects had to remember 'c' and 'k' to be matched with the probe. Matches comprised half the trials. Stimulus presentation sequence, timing and control condition were identical to that used in LTR.

The PLUS-L task was identical to PLUS except that on the non-match trials the probe was a letter in the memory stimulus set. These probes served as lures and the task was intended to evaluate executive processing; volunteers had to inhibit a prepotent response to affirm a letter they had recently viewed.

The control condition was designed to match for perceptual and motor responses. Four identical uppercase letters appeared for 0.5 s. This was followed by a shorter 0.3 s delay period prior to the appearance of a lowercase probe that matched the target in half the trials.

Each experimental run comprised six blocks of working memory tasks (two of each type counterbalanced across subjects, runs and sessions) and seven control blocks. Each volunteer underwent four experimental runs, each lasting 7 m and 9 s during each of the three sessions.

Working memory performance was assessed through accuracy at each state. Another metric used was the decline in accuracy in LTR, PLUS and PLUS-L following SD24 and SD35, relative to RW.

#### *Imaging procedure*

Images were acquired on a 3 T 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. 36 oblique axial slices with thickness 3 mm (0.3 mm gap) approximately parallel to the AC–PC line were obtained. High-resolution coplanar T1 weighted anatomical images were also obtained. A further high-resolution image was acquired using a T1 weighted 3D-MPRAGE sequence for the purpose of image display in Talairach space (Talairach and Tournoux, 1988).

Stimuli were projected onto a screen using a LCD projector and viewed by subjects through a rear-view mirror. Subjects responded by pressing buttons on a hand-held response box with the right hand. A bite-bar was used to reduce head-motion. Volunteers were monitored as data were collected on-line. They were reminded to stay alert through headphones if *three consecutive non-responses* were detected. Only a solitary reminder was issued to one of the volunteers during the RW scan, while the average number of reminders per volunteer at SD24 and SD35 was 0.42 and 0.69, respectively. At the end of each run, volunteers verbally indicated their KSS rating.

#### *Image analysis*

Functional images were processed with Brain Voyager QX 1.4 (Brain Innovation, Maastricht, Holland). Image preprocessing and registration steps have been previously described (Chee and Choo, 2004). Rigid body motion correction was performed and runs with

more than 2 mm of motion were discarded. Gaussian filtering was applied in the spatial domain using a smoothing kernel of 4 mm FWHM for individual activation maps and 8 mm FWHM for group-level activation maps. A temporal high pass filter of period 143 s was applied following linear trend removal.

Functional analysis was performed using a general linear model (GLM) with nine predictors-of-interest: three representing the levels of task difficulty for each of the scanning sessions. Due to a technical error, behavioral data were lost for one participant for the SD35 session. We also excluded behavioral and imaging data for all three sessions from two participants, one who lapsed overly frequently during the SD24 scan, and one whose Raven's Advanced Progressive Matrices scores were much below the average range (6th percentile in terms of normative data), resulting in the evaluation of data from 26 volunteers.

To evaluate the relationship between brain activation and various behavioral metrics, functional regions-of-interest (ROI) were defined by selecting regions conjointly activated (Nichols et al., 2005) in all three working memory tasks during RW at a threshold of  $P < 0.001$  (Bonferroni corrected). Parameter estimates of activation were collected for the left parietal and left prefrontal regions, shown by prior experiments to be involved in working memory, as well as from the right parietal region, the left thalamus and left anterior cingulate regions. These ROI-based estimates were subjected to a two-factor repeated-measures ANOVA using SPSS to assess the effects of task, state and their interaction. We also obtained the *average* signal change magnitude for each of the three states by averaging the activation magnitude across tasks.

Next, we compared brain activation in the different ROI between the eight volunteers who were most SD-resistant (SD-R) and the eight who were the most vulnerable (SD-V). The SD-R group consisted of the eight volunteers who demonstrated the least decline in working memory performance accuracy from RW to SD24, while the SD-V group consisted of the eight volunteers who had the greatest decline in working memory performance accuracy from RW to SD24.

Finally, we correlated average signal change with behavioral metrics that related to PVT performance and self-rated sleepiness.

To ensure the appropriateness of the control task as a baseline across states, a subset of 10 participants completed two additional runs that took place at the end of the four experimental runs. These runs consisted of three blocks of working memory (PLUS task) and three blocks of control task interleaved with fixation blocks of 21 s. During the fixation trials, subjects viewed a cross in the center of the screen and were instructed to press the response button regularly to reduce the likelihood of them falling asleep. Having explicit fixation trials allowed us to model the control task in the GLM and look for state differences, if any, in the control task across the test time points.

## **Results**

### *Behavioral data*

#### *In-scanner working memory tasks*

Performance accuracy declined significantly during the performance of the LTR, PLUS and PLUS-L working memory tasks following SD. The difference in accuracy between SD24 and SD35 was not significant. RT was longer in the various tasks following SD, the largest change being between RW and SD24. The difference in RT between SD24 and SD35 was not significant (Table 2).

Table 2

Behavioral means (and standard deviations) for accuracy and RT on the in-scanner working memory tasks during rested wakefulness (RW) and after 24 h (SD24) and 35 h (SD35) of sleep deprivation

	RW	SD24	SD35
<i>Accuracy (%)</i>			
LTR	95.2 (3.57)	86.5 (10.5) <sup>a</sup>	85.0 (9.60) <sup>b</sup>
PLUS	95.0 (4.72)	86.6 (10.6) <sup>a</sup>	84.3 (12.9) <sup>b</sup>
PLUS-L	93.9 (5.98)	82.9 (11.4) <sup>a</sup>	82.9 (12.7) <sup>b</sup>
<i>Reaction time (ms)</i>			
LTR	745 (102)	792 (96) <sup>a</sup>	769 (106)
PLUS	698 (119)	747 (94) <sup>a</sup>	746 (121) <sup>b</sup>
PLUS-L	728 (109)	773 (98) <sup>a</sup>	758 (121)

There is higher variability in performance at SD35 compared to SD24. The average coefficient of variability (CV) for accuracy at SD24 was 0.126 compared to 0.139 for SD35, while average CV for RT at SD24 was 0.124 compared to 0.153 for SD35.

None of the comparisons between SD24 and SD35 were statistically significant.

<sup>a</sup> Significant difference between RW and SD24 at  $P < 0.05$  using paired  $t$  test.

<sup>b</sup> Significant difference between RW and SD35 at  $P < 0.05$  using paired  $t$  test.

The difference in RT change between RW and SD24 for SD-R and SD-V was also significant ( $t(14) = 2.80, P = 0.01$ ), reflecting a larger RT decline for the SD-V group compared to the SD-R group (Table 3).

*M-PVT and sleepiness scales*

Both M-PVT performance metrics – RT difference between slowest and fastest RT and number of lapses – varied across time of day during the SD session (Fig. 3). Averaging data from 2300 h to 1900 h the following day, there were no significant differences in M-PVT performance between the SD-R and SD-V groups, although the SD-R group showed a trend towards better performance on both metrics of the M-PVT (Fig. 3). Variation in M-PVT performance also paralleled subjective sleepiness (compare Figs. 3 and 4). One interesting observation was the extent to which sleepiness scores were influenced by lying in the scanner (Fig. 4). This was true at RW and more impressive during SD and is in agreement with prior observations regarding the influence of posture on EEG activity and PVT performance (Caldwell et al., 2003).

Table 3

Performance of the SD-R and SD-V groups on the in-scanner working memory tasks during rested wakefulness (RW), after 24 h (SD24) and after 35 h (SD35) of sleep deprivation

	Sleep deprivation resistant (SD-R)			Sleep deprivation vulnerable (SD-V)		
	RW	SD24	SD35	RW	SD24	SD35
<i>Accuracy (%)</i>						
LTR	94.5 (2.93)	95.3 (2.89)	91.4 (4.88)	94.8 (4.85)	74.2 (7.13)	75.5 (9.30)
PLUS	95.8 (4.72)	96.6 (3.14)	92.0 (11.9)	95.1 (3.85)	74.0 (4.02)	75.0 (12.1)
PLUS-L	94.3 (4.82)	93.2 (4.69)	92.9 (7.68)	93.5 (7.59)	69.5 (7.30)	73.2 (11.6)
<i>RT (ms)</i>						
LTR	734 (127)	742 (113)	725 (154)	789 (111)	872 (60.8)	822 (69.3)
PLUS	684 (138)	689 (105)	669 (149)	714 (124)	828 (59.2)	812 (67.7)
PLUS-L	718 (140)	713 (105)	701 (172)	743 (114)	843 (88.8)	826 (68.4)

Both groups comprised eight subjects.

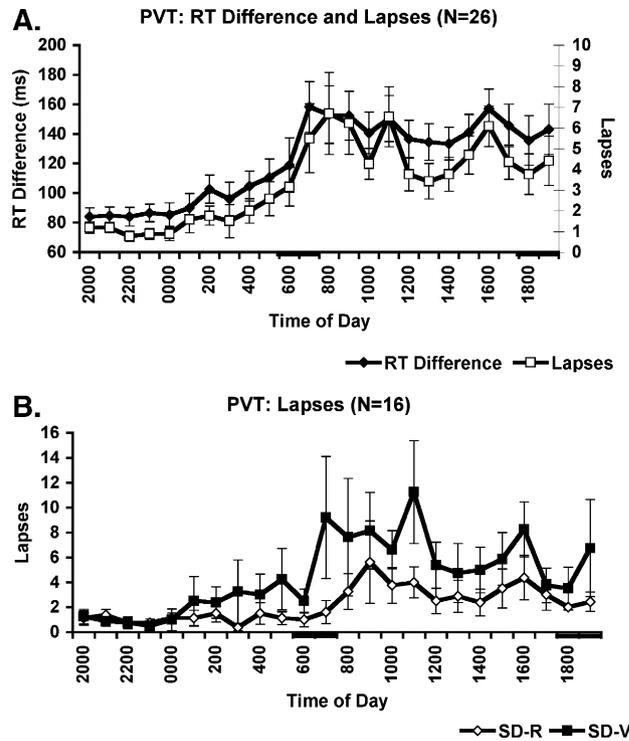


Fig. 3. M-PVT data from 26 study subjects showing the difference between the 90th and 10th percentile RT and the number of lapses across the study period (A). The sleep-deprivation resistant group (SD-R) had fewer lapses compared to the sleep-deprivation vulnerable group (SD-V) (B). Note that from 0600 h to 0800 h and again in the evening, data collection was incomplete as M-PVT was not conducted in the scanner.

*Imaging findings*

As in our previous implementation of the LTR/PLUS task (Chee and Choo, 2004), there was a strong effect of task on brain activation within the network of areas recruited when working memory is engaged (Table 4). This main effect was evident across the three tasks and at all three time-points tested. The 3 (state: RW, SD24, SD35) by 3 (task: LTR, PLUS, LURE) repeated-measures ANOVAs, conducted separately for each ROI, indicated a significant effect of task in all five ROIs investigated, namely the left lateral prefrontal region (BA 9, 44), anterior cingulate, bilateral superior parietal regions (BA 7) and the left thalamus.

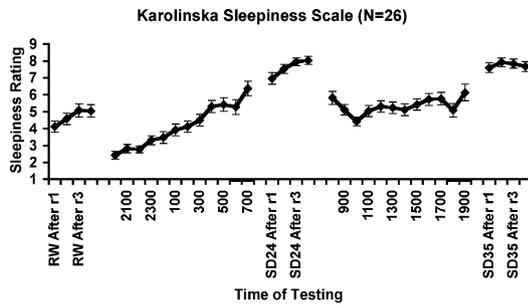


Fig. 4. Subjective sleepiness as measured by the Karolinska Sleepiness Scale (KSS) recorded outside and within the scanner. The black bars on the time axis indicate that partial out-of-scanner data were obtained from 0600 h to 0800 h and in the early evening when volunteers were being scanned. Ratings obtained following normal sleep are marked RW. The data points for RW were obtained in counterbalanced fashion although for purposes of display we plotted RW as always preceding SD.

The ANOVAs indicated a significant effect of state only in the superior parietal region (effect in left hemisphere more prominent) and the left thalamus (Table 4). Further analyses indicated significant differences between RW and SD24 as well as between RW and SD35 (reduced activation following SD compared to RW) for all three regions, with parallel effects for all three working memory tasks. However, despite the trend to lower activation at SD35 (Fig. 5), no significant differences were found between SD24 and SD35 for any of the regions in any of the tasks.

#### Relationships between working memory performance and brain activation

Significant correlations between in-scanner working memory performance and brain activation were observed in the left parietal region (Fig. 5). Activation in this region at RW and at SD24 correlated with average performance accuracy at SD24 ( $r = 0.43$ ,  $P = 0.03$  and  $r = 0.44$ ,  $P = 0.03$ , respectively), as well as the decline in performance from RW to SD24 ( $r = -0.47$ ,  $P = 0.02$  and  $r = -0.44$ ,  $P = 0.03$ , respectively). Left parietal activation at SD35 correlated with performance decline from RW to SD35 ( $r = -0.42$ ,  $P = 0.04$ ). When the top 8 (SD-R) and bottom 8 performers (SD-V) were compared, there were significant differences in activation in this region at both RW ( $t(14) = 2.45$ ,  $P = 0.03$ ) and SD24 ( $t(14) = 2.18$ ,  $P = 0.05$ ). Better performers activated the parietal region more than weaker performers (Fig. 6).

For the left prefrontal region, significant correlations were observed between working memory performance decline at SD24 and left prefrontal activation at RW ( $r = -0.44$ ,  $P = 0.03$ ; Fig. 5). Left prefrontal activation at SD35 also correlated with working memory

performance decline at SD35 ( $r = -0.42$ ,  $P = 0.03$ ). When the SD-R and SD-V groups were compared, a significant difference was present only for activation at RW ( $t(14) = 2.46$ ,  $P = 0.03$ ), again with higher activation for the better performing group (Fig. 6).

Activation in the right parietal, anterior cingulate and left thalamus was similarly higher in the SD-R compared to the SD-V group. However, the effects were less robust and the correlations between activations and performance markers did not reach significance.

#### Relationships between the M-PVT metrics, sleepiness scores and brain activation

Two M-PVT metrics of sleep deprivation vulnerability were computed by averaging mean RT difference and number of lapses (see above) for all time points between 2300 h and 1900 h. There were no significant correlations between these M-PVT metrics obtained outside the scanner and fMRI brain activation profiles during WM.

The averaged KSS score obtained in-scanner (collapsed across the ratings for each of four runs) at SD24 was inversely correlated with left parietal activation at SD24 ( $r = -0.40$ ,  $P = 0.05$ ). KSS score at SD35 similarly correlated with left parietal activation at SD35 ( $r = -0.40$ ,  $P = 0.05$ ). In contrast to Drummond et al. (2000), we did not find any correlations between sleepiness ratings and prefrontal activation at either of the two SD states.

#### Effect of time on task

Given the relatively long duration of the working memory experiment (total task time 28 min 36 s), we evaluated the potential effect of time on task by comparing the activation data obtained from the first half of each experiment (first two runs) with that obtained from the second half (third and fourth runs) by modeling the imaging data in two separate groups. We did not find a material effect of time-on-task in the prefrontal or parietal areas. However, motion artifact was more pronounced in later runs.

#### Effect of baseline task used

In this study, all activations in the experimental tasks were relative to the control task that acted as the baseline. It is possible that the lack of state effect in some regions might be due to differences in the control task activation across states in these regions, which could potentially wash out experimental differences. Additional runs including fixation trials in a subset of participants helped evaluate this possibility. When the control tasks were modeled explicitly, we did not find any differences in activation between states in the predefined ROIs. This also

Table 4  
State and task effects in the various ROI

ROI	X	Y	Z	State, $F(2,50)$	Task, $F(2,50)$	State $\times$ task, $F(4,100)$
Left prefrontal	-39	7	31	2.40	29.9*	0.51
Left parietal	-27	-64	37	11.2*	23.6*	0.58
Right parietal	30	-55	34	9.97*	14.9*	0.38
Left thalamus	-15	-22	13	4.29*	31.2*	1.87
Left anterior cingulate	-5	-1	55	2.77	14.6*	0.11

\* Indicates significance at  $P \leq 0.01$ .

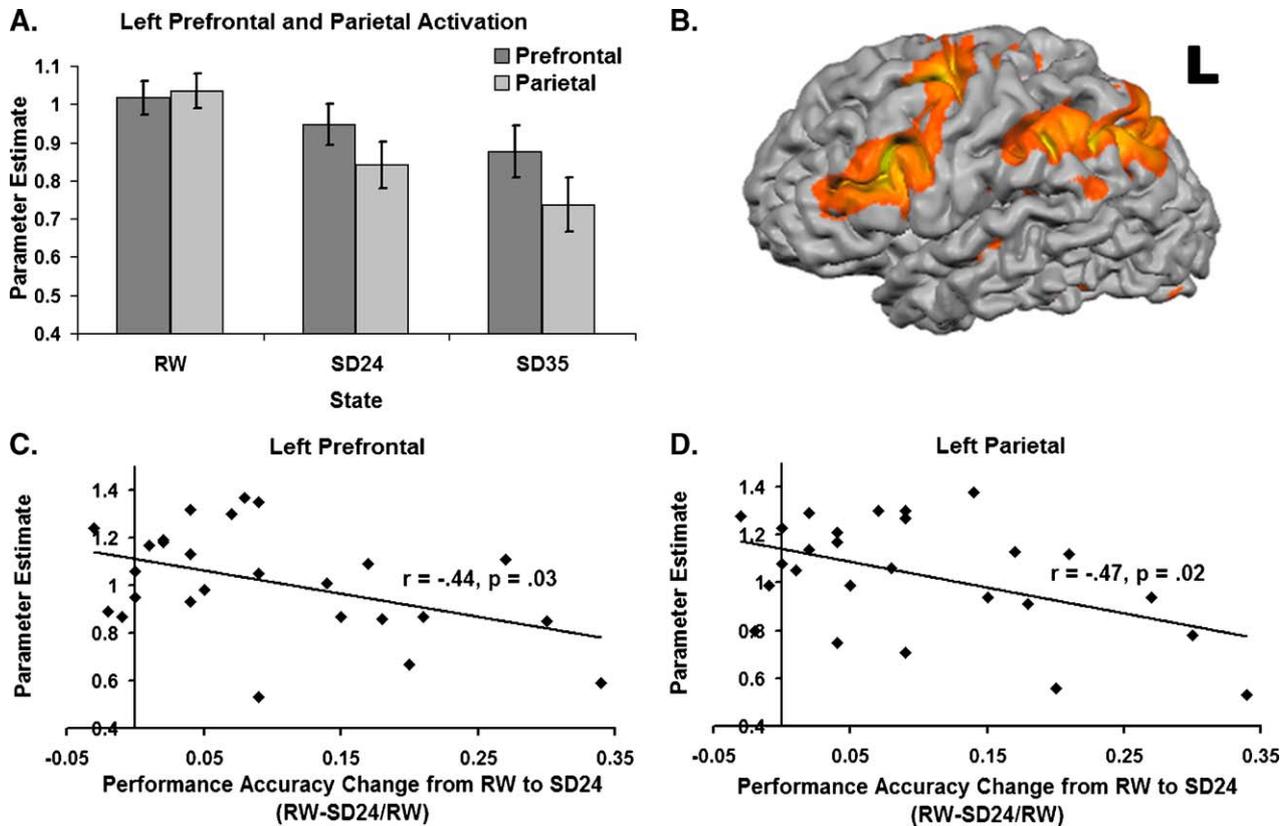


Fig. 5. (A) Average activation magnitude in the left prefrontal and left parietal regions at each test period. (B) Regions in the left hemisphere activated by all three working memory tasks; threshold  $P < 0.001$  corrected. (C) Brain activation at RW for in the left prefrontal (D) parietal regions both correlate with change in performance accuracy from RW to SD24.

suggests that the state effects seen in the experimental tasks were not a mere reflection of differences in the baseline control tasks.

## Discussion

*Imaging findings at SD24 and SD35 differ from RW but do not differ significantly from one another*

The in-scanner effects on sleepiness were pronounced at both SD24 and SD35 relative to RW. As such, lying in the scanner provided constant conditions that unmasked (accentuated) the effects of the homeostatic sleep drive at SD24 and SD35. This is ideal for comparing the effects of elevated sleep drive at the two circadian phases (Van Dongen and Dinges, 2005). Replicating SD effects at two different circadian phases increases confidence that the brain changes are due to the elevation of homeostatic sleep drive.

Although the homeostatic drive for sleep is less at SD24 compared to SD35, the circadian drive for waking at SD24 is also at a lower phase (nearer to the circadian nadir) compared to the phase at SD35 (which is closer to the circadian peak). The interaction of these effects could contribute to the finding of similar levels of behavioral cognitive impairment (Van Dongen and Dinges, 2005) as well as brain activation. There was only a non-significant trend towards lower activation of fronto-parietal areas engaged in working memory performance at SD35 compared to SD24. Thus, the effect of SD on brain activation can be regarded as comparable at the two test times while significantly different from

RW. Our findings suggest that factors other than time-of-testing are responsible for the differences observed in existing neuroimaging studies of cognition following SD. Task-related differences are one potential source for these differences (Drummond and Brown, 2001). Another possibility is that given the relatively small numbers of volunteers recruited in prior studies, the chance selection of either SD vulnerable or SD resistant subjects (Van Dongen et al., 2004) could have skewed results.

*Correlations between fronto-parietal activation at RW and working memory performance following sleep deprivation*

We found greater activation in left fronto-parietal regions after normal sleep to be associated with better-preserved working memory performance following SD (Caldwell et al., 2005; Mu et al., 2005a). Parietal activation at RW could account for approximately 20% of the variance in SD-induced performance decline.

All published imaging studies on working memory in the context of SD have shown decline in parietal activation following SD (Bell-McGinty et al., 2004; Chee and Choo, 2004; Habeck et al., 2004; Caldwell et al., 2005; Choo et al., 2005; Mu et al., 2005a), whereas results for the frontal lobe have differed among studies. As we had counterbalanced the order of the RW and SD sessions across participants and ensured that all our subjects were highly practiced on the working memory task *before* scanning, it is unlikely that reduction in parietal activation following SD was due to a learning or practice effect (Peterson et al., 1998, 1999).

The superior parietal region identified in the present study showed an effect of task, implying that it is engaged during

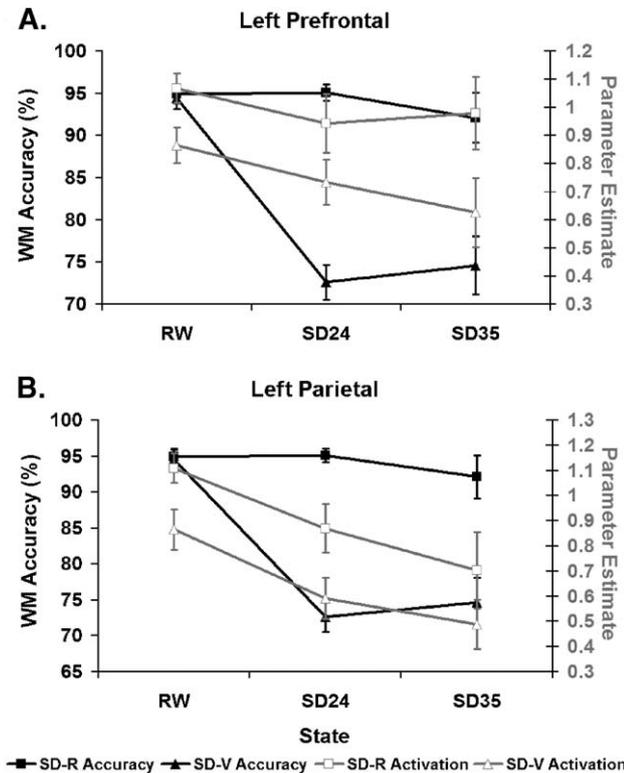


Fig. 6. Plots depicting the change, across scanning periods, in frontal and parietal activation in relation to working memory accuracy, for the SD-R and SD-V groups. The group differences in activation were significant as were differences between activation at RW and SD 24 conditions (see text for details). Although there was a trend towards a smaller decline in activation following SD for the SD-R group compared to the SD-V group, the state by group interaction was not statistically significant.

working memory operations. However, our study was not designed to uncover the specific subprocess within working memory that is sensitive to sleep deprivation. The parietal region highlighted in the present study is superior to that associated with phonological rehearsal/storage (Paulesu et al., 1993; Smith et al., 1998) revealed in a prior SD study (Habeck et al., 2004), and we posit that it might be related to the maintenance of attention (Kastner and Ungerleider, 2000; Fan et al., 2005). Delineating the specific neural network predictive of performance decline following SD is of interest because different neurotransmitter systems may be involved in attentional subprocesses like alerting and orienting (Davidson and Marrocco, 2000). As these might be potential targets for modulating performance following SD, knowledge of how each network responds to SD would be helpful.

The relationship between parietal activation at RW and working memory performance fits the ‘cognitive reserve hypothesis’, which was originally developed in the context of aging studies. This theory attributes better cognitive resistance to SD to having more cognitive resources to begin with or having the capacity to engage alternative neural resources as needs arise (Stern, 2002). We postulate that there may be a trait like, predetermined manner in which the parietal lobes influence task performance during SD. In contrast to the data obtained from the parietal lobes, the predictive value of prefrontal activation following normal sleep to performance following SD is less strong even though we found supportive correlations. This is because the engagement of the

frontal regions during task performance following SD is more variable. Prior imaging studies have shown that the magnitude of frontal activation following SD may relate to performance compensation in this state (Chee and Choo, 2004; Drummond et al., 2004). We did not replicate the finding of compensatory over-activation of the prefrontal region during SD, although there is a suggestion of compensation in the SD35 data where SD resistant individuals showed a non-significant trend to higher prefrontal activation.

The relationship between prefrontal cortical activation and working memory performance has also differed across studies performed outside the context of SD. In some reports, lower prefrontal activation has been linked to better behavioral performance (Smith et al., 2001). Studies linking lesser activation to better performance attribute the relationship to ‘greater efficiency’ of task performance (Egan et al., 2001; Rypma et al., 2002; Callicott et al., 2003). Other studies show that more competent individuals – those with higher working memory spans (Osaka et al., 2004), fluid intelligence (Gray et al., 2003), linguistic competency (Chee et al., 2004) or better task performance (Pessoa et al., 2002) – engage the prefrontal region to a greater extent. These studies attribute their findings to the greater recruitment of attentional resources (Gray et al., 2003) or engagement of superior strategies (Osaka et al., 2004) during task performance by more competent individuals.

Given these considerations, it would be premature to conclude that the relationship between higher prefrontal activation at RW and cognitive resilience to SD corresponds with ‘greater cognitive reserve’.

#### *Lack of correlation between brain activation involved in working memory and performance in M-PVT*

Despite the temporal relationship between M-PVT and working memory performance behaviorally, there was no correlation between M-PVT performance metrics and measures of brain activation, at least in regions involved in working memory. In contrast, a recent imaging study (Drummond et al., 2005) reported relationships between traditional PVT performance responses and activation/deactivation of the ‘default network’ (Raichle et al., 2001) in midline frontal regions. As such, the present data are consistent with behavioral findings suggesting that individuals show different patterns of vulnerability to performance in different cognitive domains (Frey et al., 2004; Van Dongen et al., 2004). These different cognitive domains might have imaging correlates that require systematic studies using different cognitive tests.

#### **Acknowledgments**

This work was supported by NMRC Grants 2000/0477, BMRC Grant 014, DMERI, the Shaw Foundation and AFOSR FA9550-05-1-0293 (DFD). Joanna Sze and Shen Li-Juan collected and preprocessed the imaging data. We thank the editors and three anonymous reviewers for their helpful comments.

#### **References**

Akerstedt, T., Gillberg, M., 1990. Subjective and objective sleepiness in the active individual. *Int. J. Neurosci.* 52, 29–37.

- Bell-McGinty, S., Habeck, C., Hilton, H.J., Rakitin, B., Scarmeas, N., Zarahn, E., Flynn, J., DeLaPaz, R., Basner, R., Stern, Y., 2004. Identification and differential vulnerability of a neural network in sleep deprivation. *Cereb. Cortex* 14, 496–502.
- Caldwell, J.A., Prazinko, B., Caldwell, J.L., 2003. Body posture affects electroencephalographic activity and psychomotor vigilance task performance in sleep-deprived subjects. *Clin. Neurophysiol.* 114, 23–31.
- Caldwell, J.A., Mu, Q., Smith, J.K., Mishory, A., Caldwell, J.L., Peters, G., Brown, D.L., George, M.S., 2005. Are individual differences in fatigue vulnerability related to baseline differences in cortical activation? *Behav. Neurosci.* 119, 694–707.
- Callicott, J.H., Mattay, V.S., Verchinski, B.A., Marengo, S., Egan, M.F., Weinberger, D.R., 2003. Complexity of prefrontal cortical dysfunction in schizophrenia: more than up or down. *Am. J. Psychiatry* 160, 2209–2215.
- Chee, M.W., Choo, W.C., 2004. Functional imaging of working memory after 24 hr of total sleep deprivation. *J. Neurosci.* 24, 4560–4567.
- Chee, M.W., Soon, C.S., Lee, H.L., Pallier, C., 2004. Left insula activation: a marker for language attainment in bilinguals. *Proc. Natl. Acad. Sci. U. S. A.* 101, 15265–15270.
- Choo, W.C., Lee, W.W., Venkatraman, V., Sheu, F.S., Chee, M.W., 2005. Dissociation of cortical regions modulated by both working memory load and sleep deprivation and by sleep deprivation alone. *NeuroImage* 25, 579–587.
- Curtis, C.E., D'Esposito, M., 2003. Persistent activity in the prefrontal cortex during working memory. *Trends Cogn. Sci.* 7, 415–423.
- Davidson, M.C., Marrocco, R.T., 2000. Local infusion of scopolamine into intraparietal cortex slows covert orienting in rhesus monkeys. *J. Neurophysiol.* 83, 1536–1549.
- Dinges, D.F., Pack, F., Williams, K., Gillen, K.A., Powell, J.W., Ott, G.E., Aptowicz, C., Pack, A.I., 1997. Cumulative sleepiness, mood disturbance, and psychomotor vigilance performance decrements during a week of sleep restricted to 4–5 hours per night. *Sleep* 20, 267.
- Doran, S.M., Van Dongen, H.P., Dinges, D.F., 2001. Sustained attention performance during sleep deprivation: evidence of state instability. *Arch. Ital. Biol.* 139, 253–267.
- Dorrian, J., Rogers, N.L., Dinges, D.F., 2005. Psychomotor vigilance performance: a neurocognitive assay sensitive to sleep loss. In: Kushida, C. (Ed.), *Sleep Deprivation: Clinical Issues, Pharmacology and Sleep Loss Effects*. Marcel Dekker Inc, New York, NY, pp. 39–70.
- Drummond, S.P., Brown, G.G., 2001. The effects of total sleep deprivation on cerebral responses to cognitive performance. *Neuropsychopharmacology* 25, S68–S73.
- Drummond, S.P., Brown, G.G., Stricker, J.L., Buxton, R.B., Wong, E.C., Gillin, J.C., 1999. Sleep deprivation-induced reduction in cortical functional response to serial subtraction. *Neuroreport* 10, 3745–3748.
- Drummond, S.P., Brown, G.G., Gillin, J.C., Stricker, J.L., Wong, E.C., Buxton, R.B., 2000. Altered brain response to verbal learning following sleep deprivation. *Nature* 403, 655–657.
- Drummond, S.P., Brown, G.G., Salamat, J.S., Gillin, J.C., 2004. Increasing task difficulty facilitates the cerebral compensatory response to total sleep deprivation. *Sleep* 27, 445–451.
- Drummond, S.P., Bischoff-Grethe, A., Dinges, D.F., Ayalon, L., Mednick, S.C., Meloy, M.J., 2005. The neural basis of the Psychomotor Vigilance Task. *Sleep* 28, 1059–1068.
- Durmer, J.S., Dinges, D.F., 2005. Neurocognitive consequences of sleep deprivation. *Semin. Neurol.* 25, 117–129.
- Egan, M.F., Goldberg, T.E., Kolachana, B.S., Callicott, J.H., Mattay, V.S., Straub, R.E., Goldman, D., Weinberger, D.R., 2001. Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc. Natl. Acad. Sci. U. S. A.* 98, 6917–6922.
- Fan, J., McCandliss, B.D., Fossella, J., Flombaum, J.I., Posner, M.I., 2005. The activation of attentional networks. *NeuroImage* 26, 471–479.
- Frey, D.J., Badia, P., Wright Jr., K.P., 2004. Inter- and intra-individual variability in performance near the circadian nadir during sleep deprivation. *J. Sleep Res.* 13, 305–315.
- Frey, D.J., Badia, P., Wright Jr., K.P., 2005. Analysis of inter- and intra-individual variability. *J. Sleep Res.* 14, 205–206.
- Graw, P., Krauchi, K., Knoblauch, V., Wirz-Justice, A., Cajochen, C., 2004. Circadian and wake-dependent modulation of fastest and slowest reaction times during the psychomotor vigilance task. *Physiol. Behav.* 80, 695–701.
- Gray, J.R., Chabris, C.F., Braver, T.S., 2003. Neural mechanisms of general fluid intelligence. *Nat. Neurosci.* 6, 316–322.
- Habeck, C., Rakitin, B.C., Moeller, J., Scarmeas, N., Zarahn, E., Brown, T., Stern, Y., 2004. An event-related fMRI study of the neurobehavioral impact of sleep deprivation on performance of a delayed-match-to-sample task. *Brain Res. Cogn. Brain Res.* 18, 306–321.
- Kastner, S., Ungerleider, L.G., 2000. Mechanisms of visual attention in the human cortex. *Annu. Rev. Neurosci.* 23, 315–341.
- Mu, Q., Mishory, A., Johnson, K.A., Nahas, Z., Kozel, F.A., Yamanaka, K., Bohning, D.E., George, M.S., 2005a. Decreased brain activation during a working memory task at rested baseline is associated with vulnerability to sleep deprivation. *Sleep* 28, 433–436.
- Mu, Q., Nahas, Z., Johnson, K.A., Yamanaka, K., Mishory, A., Koola, J., Hill, S., Horner, M.D., Bohning, D.E., George, M.S., 2005b. Decreased cortical response to verbal working memory following sleep deprivation. *Sleep* 28, 55–67.
- Nichols, T., Brett, M., Andersson, J., Wager, T., Poline, J.B., 2005. Valid conjunction inference with the minimum statistic. *NeuroImage* 25, 653–660.
- Osaka, N., Osaka, M., Kondo, H., Morishita, M., Fukuyama, H., Shibasaki, H., 2004. The neural basis of executive function in working memory: an fMRI study based on individual differences. *NeuroImage* 21, 623–631.
- Paulesu, E., Frith, C.D., Frackowiak, R.S., 1993. The neural correlates of the verbal component of working memory. *Nature* 362, 342–345.
- Pessoa, L., Gutierrez, E., Bandettini, P., Ungerleider, L., 2002. Neural correlates of visual working memory: fMRI amplitude predicts task performance. *Neuron* 35, 975–987.
- Petersen, S.E., van Mier, H., Fiez, J.A., Raichle, M.E., 1998. The effects of practice on the functional anatomy of task performance. *Proc. Natl. Acad. Sci. U. S. A.* 95, 853–860.
- Peterson, K.M., Elfgrén, C., Ingvar, M., 1999. Learning-related effects and functional neuroimaging. *Hum. Brain Mapp.* 7, 234–243.
- Philip, P., Taillard, J., Sagaspe, P., Valtat, C., Sanchez-Ortuno, M., Moore, N., Charles, A., Bioulac, B., 2004. Age performance and sleep deprivation. *J. Sleep Res.* 13, 105–110.
- Pilcher, J.J., Huffcutt, A.I., 1996. Effects of sleep deprivation on performance: a meta-analysis. *Sleep* 19, 318–326.
- Raichle, M.E., MacLeod, A.M., Snyder, A.Z., Powers, W.J., Gusnard, D.A., Shulman, G.L., 2001. A default mode of brain function. *Proc. Natl. Acad. Sci. U. S. A.* 98, 676–682.
- Raven, J., Raven, J.C., Court, J.H., 1998. *Manual for Raven's Progressive Matrices and Vocabulary Scales*. Oxford Psychologists Press, Oxford.
- Reuter-Lorenz, P.A., Jonides, J., Smith, E.E., Hartley, A., Miller, A., Marshuetz, C., Koeppel, R.A., 2000. Age differences in the frontal lateralization of verbal and spatial working memory revealed by PET. *J. Cogn. Neurosci.* 12, 174–187.
- Rypma, B., Berger, J.S., D'Esposito, M., 2002. The influence of working-memory demand and subject performance on prefrontal cortical activity. *J. Cogn. Neurosci.* 14, 721–731.
- Smith, E.E., Jonides, J., Marshuetz, C., Koeppel, R.A., 1998. Components of verbal working memory: evidence from neuroimaging. *Proc. Natl. Acad. Sci. U. S. A.* 95, 876–882.
- Smith, E.E., Geva, A., Jonides, J., Miller, A., Reuter-Lorenz, P., Koeppel, R.A., 2001. The neural basis of task-switching in working memory: effects of performance and aging. *Proc. Natl. Acad. Sci. U. S. A.* 98, 2095–2100.
- Stern, Y., 2002. What is cognitive reserve? Theory and research application of the reserve concept. *J. Int. Neuropsychol. Soc.* 8, 448–460.
- Talairach, J., Tournoux, P., 1988. *Co-Planar Stereotaxic Atlas of the Human Brain*. Thieme Medical Publishers, New York.

- Thomas, M., Sing, H., Belenky, G., Holcomb, H., Mayberg, H., Dannals, R., Wagner, H., Thorne, D., Popp, K., Rowland, L., Welsh, A., Balwinski, S., Redmond, D., 2000. Neural basis of alertness and cognitive performance impairments during sleepiness: I. Effects of 24 h of sleep deprivation on waking human regional brain activity. *J. Sleep Res.* 9, 335–352.
- Van Dongen, H.P., Dinges, D.F., 2005. Circadian rhythm in sleepiness, alertness and performance. In: Kryger, M.H., Roth, T., Dement, W.C. (Eds.), *Principles and Practice of Sleep Medicine*, (4th ed.) W.B. Saunders, Philadelphia, pp. 435–443.
- Van Dongen, H.P., Baynard, M.D., Maislin, G., Dinges, D.F., 2004. Systematic interindividual differences in neurobehavioral impairment from sleep loss: evidence of trait-like differential vulnerability. *Sleep* 27, 423–433.