Research report

The influence of working memory load on phase specific patterns of cortical activity

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Abstract

This study investigated patterns of cortical activity associated with the temporally separated encoding, maintenance and retrieval phases of a working memory (WM) task. Eighteen healthy subjects completed a variable load version of the Sternberg Item Recognition Task. Brain regions showing activity on average across load were determined for each task phase. In addition, brain regions showing activity that increased linearly with load were determined for encoding and retrieval. Although previous fMRI studies have used event-related designs to isolate phase specific activity, this study differed in that design and analysis methods were optimized to ensure low multicollinearity between the conditions of interest: the duration of the intermediate phase (maintenance) was varied and load was selectively modeled for the encoding and retrieval phases. The brain areas showing activity on average across load for each phase combine to encompass regions identified in previous studies that have not attempted to separate phase specific activity. Encoding is associated with extensive load dependent activity, with the most robust activity in bilateral occipital and posterior parietal regions. Retrieval is associated with more selective load dependent activity, primarily in the anterior supplementary motor region and the right posterior cerebellum. The analysis strategies employed in this study could be used to further delineate the phases of WM that are most severely compromised in clinical populations with WM disturbances.
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1. Introduction

Working memory (WM) is an important cognitive system that is thought to underlie many higher order cognitive functions, including learning, planning and reasoning [2]. Neuroimaging studies aimed at understanding which brain regions subserve the specific cognitive processes underlying WM have identified both domain (visual/spatial) [10,24,27] and temporally specific (encoding/maintenance/retrieval) phases [22,35]. Studies focusing on regional specialization have shown that the prefrontal cortex (PFC) plays a preferential role in higher order cognitive functions including the manipulation of stored information during the maintenance period [8,11,29]. Posterior parietal regions appear to be involved in storage processes, left hemisphere language areas in subvocal rehearsal processes, and occipital regions in visual pattern maintenance [7,15].

The Sternberg Item Recognition Task (SIRT) [36] is a WM task that emphasizes the maintenance of information with minimal manipulation. It consists of an encoding phase during which subjects see a string of letters that they are to remember, followed by a short unfilled delay or maintenance phase during which subjects attempt to maintain the information, and finally a retrieval phase during which subjects are shown a probe and asked to indicate whether or

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not the probe is a subset of the encoded string. The assessment of phase specific activity in time-invariant multiphase tasks, such as the SIRT, poses unique design and analysis challenges. The primary challenge of separating overlapping hemodynamic responses is similar to that found in rapid event-related designs but there is the additional complexity of fixed order events that prevents the randomization of phase sequence. Ollinger et al. [25,26] have demonstrated that for time invariant designs with two variables (analogous to two phases in a single time locked trial) the inclusion of trials that omit one of the two phases for at least 25% of the total trials is effective in separating activity associated with the two phases. This strategy is equivalent to randomization in rapid event-related designs with multiple single phase trials. While this strategy is feasible for some time invariant tasks, such as the two visual space task used by Ollinger et al., it may be undesirable or unfeasible for other multiphase tasks, such as WM tasks, where the omission of one phase may affect the subject's perception of the task.

The majority of studies that have investigated how patterns of WM activity vary with memory load have emphasized maintenance related processing by employing designs where the maintenance phase is much longer in duration than either the encoding or retrieval phase [3,7]. Only a few studies have attempted to separate out the activity associated with encoding and retrieval in addition to that associated with maintenance. Rypma and D'Esposito [34] used the SIRT to study the effects of memory load on prefrontal cortex activity during each task phase. Their finding of load dependent maintenance activity is consistent with two other studies that did not directly attempt to separate out phase specific activity: Jha and McCarthy [18] who reported load dependent frontal parietal and fusiform activity during a facial delay recognition task and Cohen et al. [7] who reported prefrontal and parietal cortex load dependent activity associated with maintenance.

An additional finding by Rypma and D'Esposito [34] was that PFC activity varied with memory load during encoding as well as during maintenance. They used a series of 4-s epochs convolved with a modeled hemodynamic response function (HRF), to estimate the magnitude of the hemodynamic response (HR) to each phase separately, and minimized the risk of misattribution of late activity from encoding to maintenance by selectively modeling only the later portion of maintenance. While this strategy minimizes the risk of misattributing encoding related activity to maintenance, it has the reverse effect of increasing the misattribution of early maintenance activity (which is not modeled) to encoding, because the model of the HRF for encoding extends into the early maintenance epoch.

Manoach et al. [22] have also attempted to separate out the activity associated with each task phase of the SIRT, but unlike Rypma and D'Esposito they did not vary memory load. Manoach et al. focused on the model of the HRF used to estimate the activity associated with each phase. They correctly point out that different brain regions may have different HRs for any given cognitive process. The error associated with using the same fixed model of the HR for all voxels and all phases has the potential to create misattribution of the activity associated with one phase to a temporally adjacent phase due to poor model fit. They used finite impulse response (FIR) functions, which make no prior assumptions about the shape of the HRF, to model the activity associated with each task phase, and reported that each phase was associated with a distinct but overlapping pattern of regional activity. Encoding was associated with activity in primary visual and visual association cortices and in the ascending segment of the intraparietal sulcus. Maintenance was associated with activity in visual association areas, bilateral primary sensory and motor cortices, supplemental motor area (SMA), and in the left premotor area. Surprisingly, no PFC activity was associated with encoding or maintenance. Retrieval was associated with the most wide spread activation, including activation in the dorsolateral PFC, thalamus and basal ganglia. While the use of FIR functions removes the risk of misattribution of phase specific activity secondary to differential goodness of fit between the modeled HRF and the actual HRF for the three phases, this approach does not take into consideration the effects of multicollinearity between modeled HRFs.

In multiple regression analysis it is only the unshared variance within the predictor variables that is useful in separating the signal from each condition of interest. Minimization of multicollinearity between the modeled HRFs is one of the most important but often overlooked study design parameters that contributes to increased accuracy and efficiency in separating overlapping signals. The main concern with regard to multicollinearity is increased standard error of the beta estimates and decreased power to detect the underlying signal. The adverse consequences of multicollinearity vary across voxels depending on the relative contributions of the events of interest to the overall signal and the goodness of fit between the model and the signal. For example, in voxels that are activated by only one of two events that have correlated models, in addition to the decrease in power due to increased standard error of the beta estimates, imprecision between the estimated model and the actual signal may lead to underestimation of the signal for the causal event and misattribution of signal to the correlated non-signal generating event. In the current study we adjusted the design and statistical model parameters to minimize multicollinearity of modeled events.

The purpose of this study was to delineate the patterns of activity associated with each phase of the SIRT and to identify brain regions that show load dependent activity during encoding and retrieval.

2. Method

2.1. Subjects

Eighteen healthy people (10 female, ages 18–35, mean age 27.5) consented to participate. All participants were right-handed, had no personal history of mental illness or neurological conditions, no generalized medical conditions requiring treatment, no family history of any psychotic disorder, and no contraindication for MR scanning. All procedures complied with the university and hospital ethical requirements.

2.2. Task design

Two 10-min runs of a variable load WM task were completed. A modified version of the SIRT [36] was programmed and presented on a personal computer using Presentation software (Neurobehavioral Systems). Stimuli were projected from an LCD projector onto a screen mounted at the foot of the MRI table. An angled mirror reflected stimuli from the screen into the participants' field of view. During a single trial of this task (Fig. 1), subjects were presented for 4 s with a string of 2, 4, 6 or 8 differing consonants in uppercase which they were instructed to remember over a short delay (3, 4 or 5 s). Subjects were required to indicate if a single consonant in lowercase presented for 1 s after this delay was the same as one of the remembered letters or different from all of the remembered letters. "Same" and "different" responses were indicted via a fiber-optic response device (Lightwave Medical, Vancouver, BC). The probability of the test letter having been in the remembered string was 0.5.

A rapid event-related design was used in order to maximize the number of trials presented for each memory load condition. As each of the task phases were short in duration, non-integer multiples of TR were used and the ITI was jittered to allow the sampling period to be effectively distributed across the entire modeled response. Jittering the ITI increases the effective sampling rate by avoiding repeated sampling at intervals the same as the TR that occurs when the ITIs are integer multiples of the TR. Each task phase was modeled as a block corresponding to its duration convolved with a synthetic HR consisting of
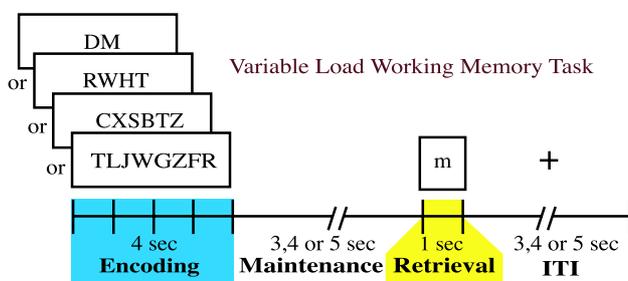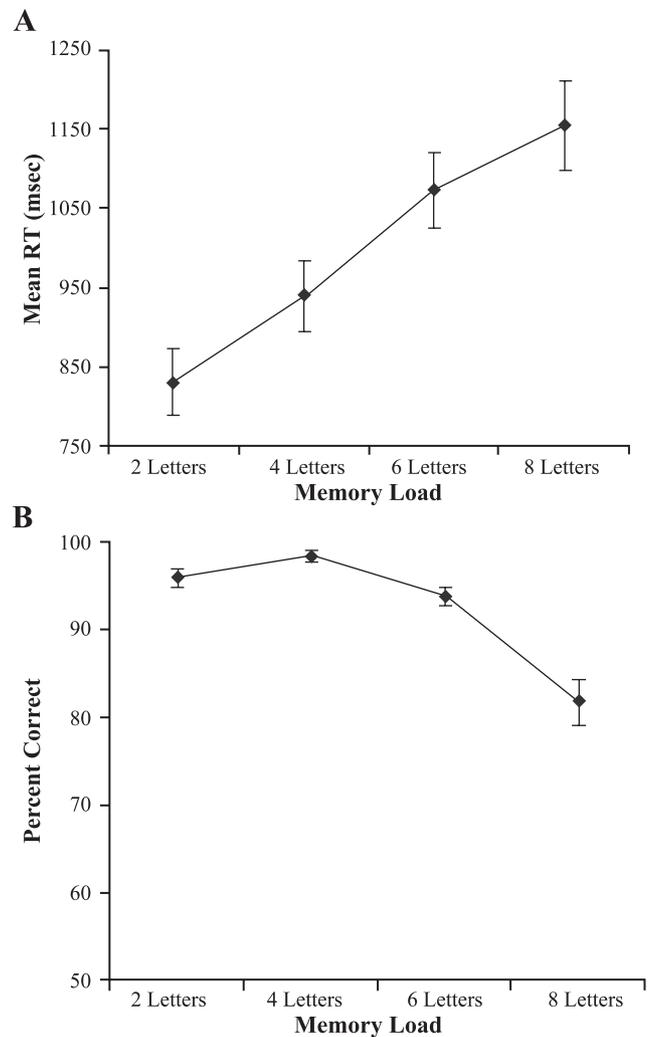

Fig. 2. Working memory performance for each memory load condition. (A) Reaction time increased linearly with memory load. (B) Response accuracy decreased when memory load was high.

two gamma function with a peak at 6 s, returning to zero at approximately 18 s with a small undershoot. These convolutions effectively result in modeled responses ranging from 22 to 18 s in duration (excluding the undershoot). As the TR used was 3 s, the positive phase of each modeled event was sampled six to seven times. Eighteen trials for each load condition allowed for between 108 and 126 data points to fit each modeled covariate.

When using rapid event-related designs it is important to ensure the conditions of interest are temporally uncorrelated in order that the unshared variance can be used to estimate the individual contribution of each condition to the overall pattern of activation. Design timing influences how well overlapping functions can be deconvolved, as the temporal pattern of onsets between conditions strongly effects the variance and the degree of correlation among these conditions [25]. The general linear model can be used to separate overlapping functions as long as the covariates are not highly correlated. To minimize the multicollinearity of

Fig. 1. Timing sequence for a single trial.

the covariates we used variable duration ITIs (3, 4 or 5 s) and randomly inserted frequent (20%) blank trials during which the word relax was presented. These two methods have previously been shown to increase the separability of overlapping functions associated with rapid event-related designs [25].

In addition, because we were primarily interested in the activity associated with encoding and retrieval, we were able to introduce two additional design and analysis features to further decrease the multicollinearity between the three within trial phases. Firstly, we jittered the duration of the maintenance phase (3, 4, or 5 s) to introduce a temporal shift between encoding and retrieval. Jittering the duration of the maintenance phase provides the same advantages as jittering the ITI. Finally, we modeled the four loads associated with encoding and retrieval separately, but modeled only the average activity across all four loads for each of the three maintenance phase durations. Modeling load for all phases results in high correlations between particular temporally adjacent covariates of the same load (maximum correlation 0.93). By selectively modeling load for encoding and retrieval but not for maintenance, the maximum correlation was reduced to 0.23.

2.3. Imaging

Echo-planar images (EPI) were collected on a standard clinical GE 1.5 T system fitted with a Horizon Echo-speed upgrade. Conventional spin-echo T_1 weighted sagittal localizers were used to view the positioning of the participant's head and to graphically prescribe the functional image volumes. Functional image volumes were collected with a gradient echo (GRE) sequence (TR/TE 3000/40 ms, 90 flip angle, FOV 24×24 cm, 64×64 matrix, 62.5 kHz bandwidth, 3.75×3.75 mm in plane resolution, 5.00 mm slice thickness, 29 slices, 145 mm axial brain coverage). This sequence is sensitive to the blood oxygen-level dependent (BOLD) contrast. Each stimulus run consisted of 196 scans (encompassing the entire brain). The first 12 s collected at the beginning of each run were discarded, to avoid variation due to T_1 saturation effects.

2.4. Data processing

Functional images were reconstructed offline. Statistical parametric mapping software (SPM99—Wellcome Institute of Cognitive Neurology, London, UK) was used for image

Table 1

Encoding: peak coordinate, *z*-score and extent of activation within each anatomical region showing significant activation

Anatomical region (Brodmann's area)[a]	Average across all loads					Linear regression with load				
	K_E	*z*-score	Peak Talairach coordinate			K_E	*z*-score	Peak Talairach coordinate		
			x	*y*	*z*			*x*	*y*	*z*
SMA (6)/CMA (32)	112	7.02	−12	2	56	46	5.19	−8	2	56
L precentral gyrus (6)	109	6.21	−48	−10	38	96	5.68	−44	−6	44
R precentral gyrus (6)	77	5.49	36	−16	56	48	5.33	40	−2	48
L inferior FG (44)	20	5.39	−52	4	18	3	4.68	−52	8	14
R inferior FG (44)	17	5.23	52	6	26	11	4.77	52	8	18
R middle FG (46)	2	4.68	32	44	28	5	4.87	36	44	24
L inferior parietal lobule (40)	26	5.23	−44	−40	42	10	5.18	−28	−48	42
R inferior parietal lobule (40)	20	5.88	32	−48	42	59	5.99	40	−36	42
L precuneus (7)	70	6.21	−24	−68	36	115	6.36	−24	−64	36
R precuneus (7)	119	6.30	28	−68	40	133	5.37	8	−56	50
R superior parietal lobule (7)						38	5.04	16	−68	52
L temporal lobe	37	5.57	−48	−62	−14	34	5.97	−48	−62	−14
R temporal lobe	28	5.75	40	−62	4	20	5.00	48	−54	−8
L occipital lobe	311	5.46	−32	−92	8	462	7.16	−16	−88	26
R occipital lobe	305	6.23	24	−92	8	418	7.06	16	96	−4
L putamen	43	6.32	−24	4	0	39	5.27	−24	8	0
R putamen	38	5.46	20	12	0	32	6.03	16	16	−4
L caudate						8	5.78	−12	20	−8
R caudate						16	5.61	12	16	−4
L globus palidus						11	4.99	−12	0	0
R globus palidus						12	5.03	16	0	4
L thalamus						15	5.83	−16	−16	−6
R thalamus						21	5.28	12	−8	12
L midbrain	14	5.57	−4	−30	−6	17	5.62	−12	12	−8
R midbrain						16	5.75	12	−16	−2
L cerebellum-posterior	39	5.39	−12	−78	−16	75	5.88	−36	−64	−18
R cerebellum-posterior	44	5.53	28	−64	−18	28	5.20	32	−60	−18

Random effects voxel level statistics, $P<0.05$ corrected for multiple comparisons.

FG=frontal gyrus.

  [a] Brodmann's areas correspond to those provided in the atlas of Talairach and Tournoux.

realignment, normalization into modified Talairach stereo-taxic anatomical space (using affine and non-linear components, as implemented in SPM99), and smoothing using a Gaussian kernel (8 mm FWHM) to compensate for intersubject anatomical differences, and to optimise the signal to noise ratio. In the Talairach coordinate frame, the origin is at the midpoint of the anterior commissure, the x axis extends from left to right, the y axis from posterior to anterior passing through the posterior and anterior com-missures, and the z axis from base of the brain to the vertex.

The BOLD response for encoding was modeled as the convolution of a 4-s box-car (beginning at the onset of the letter string to be encoded) with a synthetic HRF composed of two gamma functions [20]. Maintenance was modeled as the convolution of a 3-, 4- or 5-s boxcar (corresponding to the duration of the delay) with the synthetic HR. Finally, retrieval was modeled as the convolution of a 1-s boxcar (beginning at the onset of the test letter) with the synthetic HR. The primary function of the blank trials was to decrease the temporal correlation between trials and to increase sensitivity to differences between the modeled responses and the implicit baseline; therefore the blank trials were not explicitly modeled. A high pass filter (cut-off period 89 s) was incorporated into the model to remove noise associated with low frequency confounds (e.g. respiratory artefact). A notch filter (at the Nyquist frequency, with a period of 6 s) was also applied to remove noise associated with alternations of the applied radio frequency field. The parameters of the modeled HR were adjusted to fit the observed BOLD signal time course in each voxel, employing the General Linear Model, using SPM99.

2.5. Statistical analysis

In the first level fixed effects analysis, 11 conditions were modeled: four memory load conditions (2, 4, 6, 8 letters) for both encoding and retrieval and three maintenance conditions (3,4 or 5 s duration). In the second level mixed effects analysis, the following five contrasts were computed for each subject: (1) average encoding activity vs. implicit baseline, (2) average retrieval activity vs. implicit baseline, (3) a linear regression of encoding activity with load, (4) a linear regression of retrieval activity with load and (5) average maintenance activity vs. implicit baseline. Contrasts for the linear regressions were completed using the parameter estimates -3, -1, 1, and 3 for the 2, 4, 6 and 8 letter conditions, respectively. A one-sample t-test was computed on the individual subject contrast images (one image per subject) to determine regions of significant activation over all subjects for each of the five contrasts. A height threshold of $P<0.05$ corrected for multiple comparisons across the whole brain was used unless otherwise specified.

2.6. Post hoc region of interest (ROI) analysis

A binary mask was created of regions found to increase significantly with load during encoding (height threshold $t=6.64$, $P<0.05$ corrected for multiple comparisons, cluster extent $(k)=2158$). The number of above-threshold voxels (height threshold of $t=3.11$, $P<0.001$ uncorrected for multiple comparisons) within the load dependent encoding mask was determined for each subjects' load specific encoding and retrieval contrasts.

3. Results

3.1. Behavioral

Reaction times (RTs) were submitted to an ANOVA which revealed a main effect of load $[F(3,15)=87.24$, $P<0.001]$. Mean RTs for each load condition are shown in Fig. 2A. Post hoc t-tests confirmed that with each increase
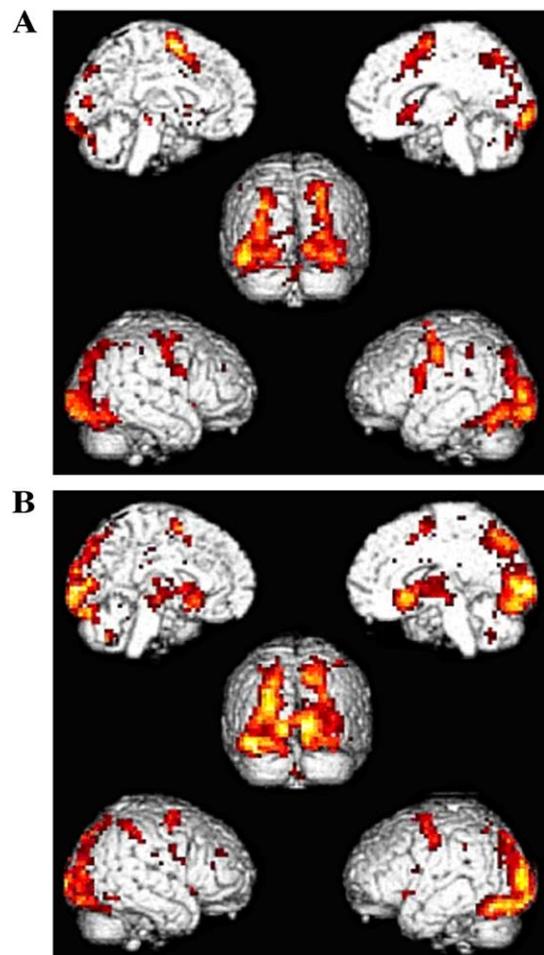

Fig. 3. Encoding phase: surface renderings of areas of significant activation. ($P<0.05$, corrected for whole brain). The majority of areas that showed significant average activity over load (A) also exhibited activity that increased linearly with load (B), including bilateral precentral, prefrontal (BA6), inferior frontal, parietal (BA 7/40), and occipital regions; see Table 1.

Table 2
Maintenance: peak coordinate, z-score and extent of activation within each anatomical region showing significant activation

Anatomical region (Brodmann's area)	K_E	z-score	Peak Talairach coordinate		
			x	y	z
SMA (6)/CMA (32)	105	5.34	−4	10	48
L precentral gyrus (6)	38	4.57	−44	−6	52
L superior FG (6)	30	4.29	−16	10	58
R superior FG (6)	16	4.37	20	10	54
L inferior(45)/ middle FG (46)	79	4.22	−48	32	20
R middle FG (46)	20	4.47	36	48	24
L parietal-precuneus (7)	24	3.92	−28	−60	40
L occipital lobe (17)	56	4.52	−20	−94	−2
R occipital lobe (17)	101	4.52	16	−90	4
L putamen (48)	19	3.96	−24	12	−8
R putamen(48)/insula(47)	26	3.92	32	24	−2
R cerebellum-posterior	29	4.50	28	−64	−24

Random effects cluster level statistics, $P<0.05$ corrected for multiple comparisons.

in memory load there was a significant increase in RT [2–4 letters, $t(17)=4.75$, $P<0.001$; 4–6 letters, $t(17)=6.70$, $P<0.001$; 6–8 letters, $t(17)=2.66$, $P<0.017$].

An ANOVA of the error rates revealed a main effect of load [$F(3,15)=16.75$, $P<0.001$]. Mean error rates for each load condition are shown in Fig. 2B. Post hoc t-tests revealed that although two-letter accuracy was not statistically different from four-letter or six-letter accuracy, there was a significant decrease in accuracy from four to six letters [$t(17)=3.83$, $P=0.001$]. In addition, performance accuracy was significantly lower for the eight-letter condition then for each of the lower memory load conditions

[2–8 letters, $t(17)=4.87$, $P<0.001$; 4–8 letters, $t(17)=6.78$, $P<0.001$; 6–8 letters, $t(17)=4.96$, $P<0.001$]. The mean number of correct responses for all loads was significantly above chance (minimum accuracy 83.3% for the eight-letter condition).

3.2. Imaging data

3.2.1. Encoding phase

The peak Talairach coordinate, z-score and extent of activation within each anatomical region in which significant activation was found is shown for both the average and the load dependent encoding analyses in Table 1. The contrast for average activation across the four memory load conditions revealed activation in a widespread network of cortical and sub-cortical regions (Fig. 3A). Significant activation was identified bilaterally in the occipital cortex, inferior parietal lobules, precuneus, temporal lobes, putamen, inferior frontal gyri and posterior lobe of the cerebellum. Bilateral activation, that was greater in the left than the right hemisphere, was identified in the precentral gyrus, SMA (extending into the cingulate motor area (CMA)) and midbrain. A small significant cluster was also found in the right middle frontal gyrus (BA 46).

As can be seen in Fig. 3B, activation increased linearly with load in the majority of areas that showed significant average encoding activity. The two contrasts showed very few differences: Activation in the right parietal lobule extended more superiorly in the load dependent contrast than in the mean contrast. The load dependent contrast revealed significant activation in some subcortical regions

Table 3
Retrieval: peak coordinate, z-score and extent of activation within each anatomical region showing significant activation

Anatomical region (Brodmann's area)	Average across all loads					Linear regression with load				
	K_E	z-score	Peak Talairach coordinate			K_E	z-score	Peak Talairach coordinate		
			x	y	z			x	y	z
SMA(6)/CMA (32)	47	5.62	4	14	44	24	6.28	0	28	36
L precentral gyrus 1° (4)	2	5.14	−40	−16	56					
L precentral gyrus (6)	4	4.91	−48	−4	8					
R inferior FG (46)						6	5.59	40	32	24
R middle FG (46)						1	4.86	32	52	16
L insula (47)	15	5.14	−36	20	−8					
R insula (47)	10	5.14	32	26	−4					
L inferior parietal lobule (40)	134	6.13	−48	−34	38					
R inferior parietal lobule (40)	15	4.99	32	−44	46					
L postcentral gyrus (2)	4	5.04	−40	−36	60					
R postcentral gyrus (48)	2	5.05	56	−18	34					
R occipital lobe (7)	2	5.01	12	−68	36					
L middle temporal gyrus (37)	13	5.41	−56	−66	4					
R inferior temporal gyrus (37)	9	5.68	48	−58	0					
Midbrain	2	5.68	−16	−12	−6					
R cerebellum-anterior lobe	36	5.87	20	−56	−20					
	11	5.81	0	−58	−8					
R cerebellum-posterior lobe	4	5.36	24	−64	−40	15	5.30	−4	−78	−16
	4	5.30	12	−70	−20	1	5.28	44	−64	−28
	3	5.07	8	−80	−30	5	4.84	36	−70	−20

Random effects voxel level statistics, $P<0.05$ corrected for multiple comparisons.
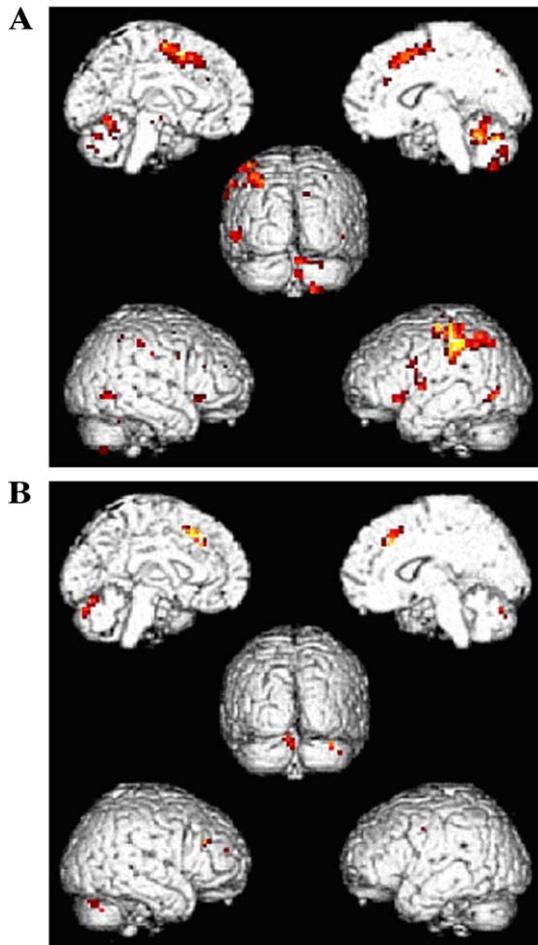
Fig. 4. Retrieval phase: surface renderings of areas of significant activation. (*P*<0.05, corrected for whole brain). (A) Average activity over load was identified in regions including the SMA/CMA, bilateral inferior parietal lobule, primary motor cortex and right cerebellum. (B) Areas of load dependent activation were confined to the anterior SMA/CMA, cerebellum and the right middle frontal gyrus; see Table 3.

including the caudate, globus palidus and thalamus which did not meet significance for the mean contrast.

3.2.2. Maintenance phase

The peak Talairach coordinate, *z*-score and extent of activation within each anatomical region in which significant activation was found is shown for the average maintenance analysis in Table 2. An inclusion criterion of *P*<0.001(uncorrected for multiple comparisons) was chosen for this phase, only clusters that reached the cluster level significance of *P*<0.05 (corrected for multiple comparisons) are reported. The contrast for average activation across the four memory load conditions revealed significant clusters centered in the left SMA/CMA extending into bilateral superior frontal regions (BA6) and the left precentral gyrus; in the left occipital lobe, extending into the right occipital lobe and right cerebellum; in the left inferior frontal gyrus (45) extending into the left middle frontal gyrus (46); and in the bilateral putamen, left precuneus and right insula.

3.2.3. Retrieval phase

The peak Talairach coordinate, *z*-score and extent of activation within each anatomical region in which significant activation was found is shown for both the average and the load dependent retrieval analyses in Table 3. The contrast for average activation across the four memory load conditions revealed significant activation in the SMA/CMA, left inferior parietal lobule, and the right anterior lobe of the cerebellum. Smaller significant clusters were also present unilaterally in the left primary motor cortex, left precentral gyrus, right occipital cortex, right inferior parietal lobule, left midbrain and right posterior lobe of the cerebellum; and bilaterally in the temporal lobe, postcentral gyrus and the insula (Fig. 4A).

In contrast to what was found for encoding, retrieval activation did not increase linearly with load in the majority of the regions that showed significant average retrieval activity (Fig. 4B). Only activity in the SMA/CMA and small clusters in the right posterior lobe of the cerebellum reached statistical significance for the load dependent contrast. The SMA/CMA activity was more anterior in the load dependent analysis than that found in the average analysis. Additional small clusters in the right inferior frontal gyrus (46) and the right middle frontal gyrus (46) did not reach statistical significance for the mean contrast, but did reach significance for the load dependent contrast.

3.2.4. Post hoc ROI analysis: load dependent encoding mask

For both encoding and retrieval the mean number of above-threshold voxels (height threshold *t*=3.11, *P*<0.001 uncorrected for multiple comparisons) within the mask of
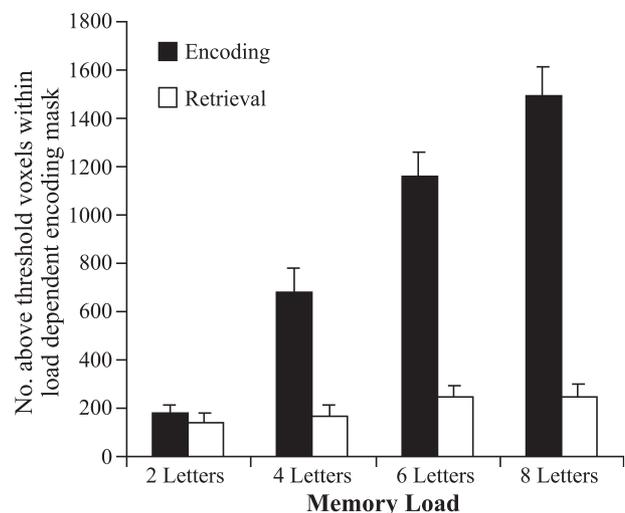

Fig. 5. Extent of encoding and retrieval activity (voxels *T*>3.11, *P*<0.001 uncorrected for multiple comparisons) found within a mask of the load dependent encoding regions (see Fig. 3B) for each memory load condition. With each increase in load, there was a significant increase in the extent of encoding activation within the mask [2–4 letters, *t*(17)=−5.95, *P*<0.001; 4–6 letters, *t*(17)=−4.74, *P*<0.001; 6–8 letters, *t*(17)=4.43, *P*<0.001]. In contrast, the extent of retrieval activity within the mask did not vary significantly with load.

load dependent encoding regions are shown for each load condition in Fig. 5. An ANOVA of the extent of encoding activation within the mask revealed a main effect of load [$F(3)=34.88$, $P<0.001$]. Post hoc t-tests confirmed that there was a significant increase in the extent of encoding activation with each increase in memory load [2–4 letters, $t(17)=-5.95$, $P<0.001$; 4–6 letters, $t(17)=-4.74$, $P<0.001$; 6–8 letters, $t(17)=4.43$, $P<0.001$] (Fig. 5). In contrast, an ANOVA of the extent of retrieval activation within the mask revealed a no main effect of load [$F(3)=1.27$, $P=0.29$].

4. Discussion

The goal of the current study was to isolate the patterns of cortical activity associated with the temporally separated encoding, maintenance and retrieval phases of a WM task and to determine which of the regions involved in encoding and retrieval exhibit activity that increases linearly with load. Other investigators have used subtraction methods to separate delay phase activity from encoding and retrieval [1,16,22]. While these methods can separate regions active during maintenance exclusive of regions active during encoding and retrieval, an equivalent method for isolating encoding activity from maintenance activity is not as straightforward, as it is not possible to invoke maintenance in the absence of encoding. Our approach was to separate the encoding, maintenance and retrieval phases with focused efforts aimed at minimizing multicollinearity.

It should be noted that the selective modelling procedure utilized in this study represents a compromise solution to the multicollinearity problem for multi-univariate analyses. The consequences of high correlation between two modeled events at any given voxel depend on the degree to which each event activates the voxel of interest. For voxels that are activated during encoding but not during maintenance, high correlation between maintenance and encoding models would lead to increased standard error of the beta estimates and increased risk of failure to detect the encoding signal (Type II error). By selectively modeling the average activity for the maintenance period we were able to obviate this concern by reducing the maximum correlation between modeled events from $r=0.93$ to $r=0.23$. However, in voxels with significant maintenance activity, the consequence of using an average activity model for maintenance includes decreasing the goodness of fit between the model and the actual signal, resulting in increased residual error, decreased statistical power and increased risk of a type II error for detecting maintenance signal. In addition, the residual delay signal may potentially be misattributed to the encoding model with which it was correlated.

In order to estimate the magnitude of the biases associated with our procedures, we assessed the effects of selective modelling of the mean activity for events with known load dependent activity with and without a model of a correlated but non-signal generating event. Inclusion of a correlated non-signal generating model ($r=0.7$) resulted in a 40% increase in the standard error, and a corresponding 40% decrease in the t-score (beta estimates were unchanged because the model predicted the signal with 100% accuracy). Modelling the mean activity instead of four separate levels of load dependence increased the power to detect the underlying signal by decreasing the standard error of the beta estimates as fewer models result in decreased multicollinearity. The net result in our simulation was a 13% reduction in the t-score relative to the value for the load condition with the highest activity and a 15%, 106% and 202% increase in t-score relative to the load conditions with lower activity. The beta estimates for the model of the correlated but non-generating event remain at or near zero, regardless of whether the load dependent models or average activity model is used. The results of our simulation show that the selective modeling of the average delay activity utilized in this study can substantially increase the power to detect encoding activity for voxels that are only activated by the delay phase, at the expense of a small decrease in the power to detect maintenance activity. There was minimal risk of misattribution of maintenance activity to the encoding phase (detailed methods and results for the simulation are available from the corresponding author).

As discussed below, the patterns of regional activity identified for each phase, are plausible in light of previous research. The areas identified for each phase combine to encompass regions previously identified in fMRI studies of WM that have not attempted to separate phase specific activity. Parametric analyses revealed that the majority of the regions activated during encoding show load dependent activity. In contrast, the majority of the regions activated during retrieval do not show load dependent activity.

4.1. Behavioral performance

As has been commonly found, response times increased significantly with each increase in memory load. Performance accuracy was significantly above chance for all memory load conditions. Although accuracy was lower for the eight-letter condition than for any of the lower memory load conditions, the percentage of correct eight-letter responses was still above chance, indicating that subjects remained engaged as task difficulty increased.

4.2. Encoding phase

During encoding the contrast for mean activity across all four memory loads revealed significant activation in a distributed network of regions involved in visual pattern processing, subvocal rehearsal and the storage of visual and verbal information. Similar patterns for encoding have been demonstrated by Rypma and D'esposito [34] and Manoach et al. [22], but the finding that these areas also show load dependent activity has not been previously

reported. The one previous study by Rypma and D'Esposito that also separated phase specific activity and assessed load dependence, used a region of interest (ROI) analysis focusing primarily on the PFC to investigate the effects of load, therefore comparisons in other brain areas are not possible. In contrast to Rypma and D'Esposito, we found only very limited load dependent activity in the PFC at our chosen threshold of $P<0.05$ after correction for multiple comparisons.

The use of a priori ROIs, such as those used by Rypma and D'Esposito [34] obviate the need to use whole brain correction and dramatically increase the power to detect differences or load dependent relationships. In light of Rypma and D'Esposito's finding, our threshold of $P<0.05$ after whole brain correction is unnecessarily stringent to detect PFC load dependence during encoding. Indeed, if our threshold is decreased to $P<0.001$ uncorrected, load dependent PFC activity is revealed. Our results indicate that while the PFC does likely show load dependent activity during encoding it is the occipital and posterior parietal regions that show the most robust load dependent activity during this phase.

Attentional mechanisms may account for bilateral occipital activity observed during encoding. Salient stimulus processing is an involuntary attentional mechanism that allows information that is most likely to be relevant to behaviour to be selected from the environment [9,28]. This attentional mechanism has been shown to involve relevant sensory association areas, a right lateralized network of brain areas and bilateral occipital regions [12,13]. Many of the regions which exhibited load dependent activity during encoding were those previously implicated in salient stimulus processing, including bilateral occipital regions, the SMA, premotor cortex, bilateral precuneus and the right cerebellum. The observed linear increase in activity with load in extensive occipital regions suggests that the role of the occipital cortex includes memory or attentional aspects in addition to perceptional aspects.

Alternatively, the load dependent increase in activity may have been related to perceptual demand as letter string length also varied with memory load in the current study. The observation that occipital activation increases with memory load, in an *n*-back fMRI study in which WM load for faces was varied but perceptual and motor demands were held constant [14], suggests that the bilateral increase in occipital cortex activation seen in the current study may also be due to increasing memory or attentional demands. Furthermore, in a separate analysis of this data set using constrained principal component analysis [5], the activity in the occipital cortex/precuneus and anterior cingulate cortex (a region that has been associated with attention [4,6]) load onto a common component, indicating that they are functionally connected by way of a common brain system. This finding lends further support for the proposed role of the occipital cortex in a brain system mediating attentional processes.

4.3. Maintenance phase

Our finding of significant activation during the maintenance phase in bilateral lateral prefrontal and left lateralized parietal regions is consistent with most previous functional imaging, single neuron recording and lesion studies that have all implicated these areas in the maintenance of memory across a short delay [7,19,31]. In contrast, Manoach et al. [22] found no significant activation in the PFC during maintenance using a study design very similar to ours. This discrepancy may be accounted for by the different contrasts or definitions used in the studies. Our study defined maintenance activity as the magnitude of the estimated response during the period encompassed by this phase. Manoach et al. utilized a relatively novel definition of maintenance phase activity: the average activity across all time points (including the encoding and retrieval periods) for trials with a 4-s maintenance period minus the average activity across all time points for trials with no maintenance periods. Manoach et al.'s finding of no significant activity in the PFC for this comparison may be the result of similar magnitude and extent of activation in the PFC in both trials. This interpretation is consistent with our finding and Rypma and D'Esposito's [34] finding that the PFC is activated during encoding as well as during maintenance.

In addition to prefrontal and parietal regions, we also identified significant clusters of activity in left hemisphere premotor and supplementary motor areas, the left inferior frontal gyrus (regions implicated in verbal rehearsal) and in bilateral occipital regions during the maintenance phase. The finding that the occipital cortex is still active after the visual display has disappeared, adds support to the notion that the occipital cortex may contribute to abstract memory representation in addition to its role in visual perception.

4.4. Retrieval phase

During retrieval, the contrast for mean activity across all four memory loads revealed significant activation in regions previously implicated in response preparation and execution [17,32,33], including the SMA/CMA, the left postcentral gyrus, left primary motor cortex and right cerebellum. Interestingly, memory load appears to exert a greater effect during encoding than it does during retrieval, whereas the majority of regions active on average during encoding exhibited significant load dependent activity, very few of the regions active on average during retrieval exhibited significant load dependent activity. This is consistent with Rypma et al.'s [35] report that memory load exerts a greater effect on prefrontal activation during encoding than it does during retrieval. The current result extends this earlier finding by demonstrating that memory load exerts a greater effect during encoding than during retrieval in many brain regions in addition to prefrontal regions.

In the current study, as in Monoach et al.'s recent study [22], SMA activity was identified during all three task phases, whereas primary motor activity was restricted to retrieval. The finding that retrieval related activity in the SMA/CMA is load dependent, whereas retrieval related activity in the primary motor region is load independent, is also consistent with previous studies. In an fMRI study of memory retrieval and search processes by Pollman et al. [30] the amplitude of the BOLD response was found to increase with memory load in the SMA but not in the primary motor cortex. Interestingly the region of the SMA/CMA that we identified as showing load dependent activity was more anterior to the region of the SMA/CMA that showed load independent activity. The anterior SMA, which receives inputs from the PFC and CMA has been implicated in the higher order function of movement decision making, whereas the more posterior SMA, which receives inputs from and projects to the primary motor cortex, has been implicated in the lower order function of motor execution [23].

Leuthold and Jentzsch [21] completed an event-related potential (ERP) study exploring the brain regions involved in preprogramming and execution of movements. They identified a medial dipole, associated with the anterior SMA/CMA, which exhibited activity that increased with the extent of advance motor preparation, unlike a later onset lateral dipole, which was associated with the lateral premotor and primary motor cortex. Based on these findings and those of earlier electrophysiological studies [37], Leuthold and Jentzsch proposed that the SMA/CMA are responsible for the more abstract, higher order and load dependent aspects of response preparation including assembly and selection of movement programs, whereas the lateral premotor cortex and the primary motor cortex are responsible for lower order and load independent aspects of response preparation including more muscle-specific response preparation tasks.

The identification of anterior SMA/CMA load dependent activity, with premotor and primary motor cortex load independent activity in the current study, confirms the anatomical substrates suggested by electrophysiological studies and supports the notion that that regions responsible for higher motor functions, such as assembly and selection of a motor program, exhibit load dependent activity, whereas regions involved in lower order motor functions, such as response execution, exhibit load independent activity.

5. Conclusions

By selectively modeling memory load for distinct phases (encoding and retrieval), and varying the duration of the intermediate phase (maintenance), we were able to isolate the patterns of cortical activity associated with the temporally separated encoding, maintenance and retrieval phases of a WM task. Parametric analyses of the encoding and retrieval phases revealed extensive load dependent activity during encoding and more selective load dependent activity during responding.

The analysis strategies employed in this study could be used to further delineate the phases of working memory that are most severely compromised in clinical populations that have known working memory disturbances. These studies have the potential to help in further focusing rehabilitation efforts to assist individuals with working memory deficits.

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