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fMRI BOLD response to increasing task difficulty during successful paired associates learning

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Abstract

We used functional magnetic resonance imaging (fMRI) to assess cortical activations associated with increasing task difficulty (TD) in a visuospatial paired associates learning task. Encoding and retrieval were examined when 100% successful retrieval of three, four, or six object–location pairs had been attained (thus ensuring that performance was matched across subjects). As memory load increased, in general, the number of attempts taken to achieve 100% successful retrieval increased, while the number of trials correctly completed on the first attempt decreased. By modelling parametric variations in working memory load with BOLD signal changes we were able to identify brain regions displaying linear and nonlinear responses to increasing load. During encoding, load-independent activations were found in occipitoparietal cortices (excluding the precuneus for which linear load dependency was demonstrated), anterior cingulate, and cerebellum, while linear load-dependent activations in these same regions were found during retrieval. Nonlinear load-dependent responses, as identified by categorical contrasts between levels of load, were found in the right DLPFC and left inferior frontal gyrus. The cortical response to increasing cognitive demands or TD appears to involve the same, rather than an additional, network of brain regions "working harder."

Introduction

Although much is known from functional imaging experiments about regional activity in the brain accompanying particular cognitive operations, very little is known about the way in which the brain responds to increasing task demands within a particular domain of cognition. In both theoretical and practical terms, it is important to understand the relationship between increasing task difficulty (TD) and blood flow (PET) or BOLD response (fMRI) given that a manipulation of TD occurs in most functional imaging studies. Studies in which contrasts are made between active and control conditions inherently vary TD or task complexity (e.g., a mnemonic task of encoding pictures is cognitively more demanding than a perceptual task of attending to visual noise patterns). When functional imaging is used to assess the impact of age, disease, or drugs upon cognitive processing there is an even greater need to distinguish between changes in brain function or organization and changes that reflect responses to variations in TD. Such studies make the assumption that neuroimaging is measuring changes in blood flow or the BOLD response associated with the specific effect of age, disease, or drug, whereas changes could be related to or mediated by the more general effect of TD. Without understanding the impact of TD per se, it is difficult to interpret such between group imaging data.

In a review of 107 PET studies which had reported anterior cingulate activation, Paus et al. (1998) reported an effect of TD or task complexity on blood flow in the anterior cingulate gyrus, particularly in the paralimbic area of the supracallosal anterior cingulate. These authors found a positive correlation between working memory (WM) and TD, so that the greater the TD, the more likely WM was to have been engaged. They concluded that increased arousal during

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the performance of difficult or attentionally demanding tasks, together with WM demands, mediated the effects of TD on blood flow in the supracallosal anterior cingulate. Duncan and Owen (2000) reviewed the location of activations found in a range of tasks that had varied cognitive demand and were able to identify a common network of regions thought to be involved in TD. Middorsolateral prefrontal (in the region of the middle and posterior inferior frontal sulcus), midventrolateral prefrontal (dorsal and anterior to the Sylvian fissure, extending to the anterior insula via the frontal operculum), and dorsal anterior cingulate cortices were all activated under conditions of increased cognitive load in studies of response conflict, task novelty, WM (in tasks varying number of items to be maintained and delay), perceptual degradation, and episodic retrieval. The recruitment of this particular activation network appears to be both task independent (found across different cognitive domains) and stimuli independent (found across different stimulus types and modalities).

The response to increasing cognitive demands or TD may involve either the same network of brain regions "working harder," recruitment of a different or additional "compensatory" network, or a combination of the two. All three patterns have been suggested by existing studies. Reports of a monotonic or linear relationship between signal change and memory load in bilateral frontoparietal regions in *n*-back WM studies (e.g., Braver et al., 1997; Carlson et al., 1998; Jonides et al., 1997) suggest that increasing TD is supported by the same network of brain regions "working harder." Alternatively, an increase in activation in right frontoparietal regions during a complex matching-to-sample task compared to simple matching-to-sample, but left frontoparietal activations when comparing both types of matching-to-sample with a control task (Klingberg et al., 1997) might suggest that *different* "compensatory" brain regions are recruited in order to respond to increasing TD in these tasks. During maintenance of six letters vs one letter in WM, greater activation of the *same* temporal-parietal regions identified in a comparison between three letters and one letter, together with *additional* frontalparietal activations (Rypma et al., 1999) suggests that both recruitment of additional "compensatory" brain regions and an increase in activation in an existing network support increasing TD.

In the present study we manipulated TD by parametrically varying memory load in an object–location paired associate memory task, specifically examining the effect of increasing cognitive demand upon encoding (or learning) and retrieval processes. By modelling parametric variations in working memory load with BOLD signal changes we were able to identify brain regions displaying linear and nonlinear responses to increasing load. During the encoding or paired-associate learning phase participants were required to remember *what* object appeared *where,* while during retrieval participants were required to perform a two-alternative forced-choice recognition task in each object–location. Memory loads for each individual were determined by offline testing prior to scanning in order to ensure that TD was matched to each participant's level of ability. This is in contrast to previous studies in which prespecified levels of memory load remain static across subjects, with individual responses to TD not being accounted for. The influence of TD upon encoding and retrieval during paired associate learning was examined when 100% successful performance had been achieved (and so performance was matched across subjects). This ensured that regions responding to increasing cognitive demands were not confounded by the failing performance that typically accompanies increasing TD.

While the effect of increasing cognitive load in WM, either by manipulating the number of trials to be monitored and updated (e.g., in an *n*-back task), the number of items to be maintained, or the time period over which items should be maintained has been previously studied, little is known about the effect of increasing TD during successful learning in a paired associate task. Following the findings of bilateral frontoparietal increases in activation with increasing memory load in *n*-back WM studies and prefrontal and anterior cingulate cortical activations in tasks that manipulate TD, we expected to see increasing activation within bilateral frontal and parietal cortices and the anterior cingulate gyrus in response to an increasing number of associative object–location pairs.

Materials and methods

Participants

Eleven young adults (six female, five male, mean age = 28.7 years, age range = 20–35 years, mean years education = 17.9) and 12 older adults (six female, six male, mean age = 64.4 years, age range = 59–71 years, mean years education = 14.8) participated in the study. Initially, the aim of this study was to examine the effects of age and task difficulty upon encoding and retrieval processing. However, in subsequent analyses of fMRI data no age differences were found and so, hereafter, subjects will be treated as one group ($n = 23$). All volunteers were healthy, right-handed, and without a history of medical or psychiatric problems. All volunteers were native English speakers and provided written informed consent prior to testing. Normal scores on the Beck Depression Inventory (Beck et al., 1961), Geriatric Depression Scale (Yesavage et al., 1983), Mini-Mental State Examination (Folstein et al., 1975), and National Adult Reading Test (Nelson and Willison, 1991) were attained by all participants. This study was approved by the Cambridge Local Research Ethics Committee.

Materials and procedure

Stimuli for the Visuospatial Encoding and Retrieval Task (VERT) consisted of six white squares against a black

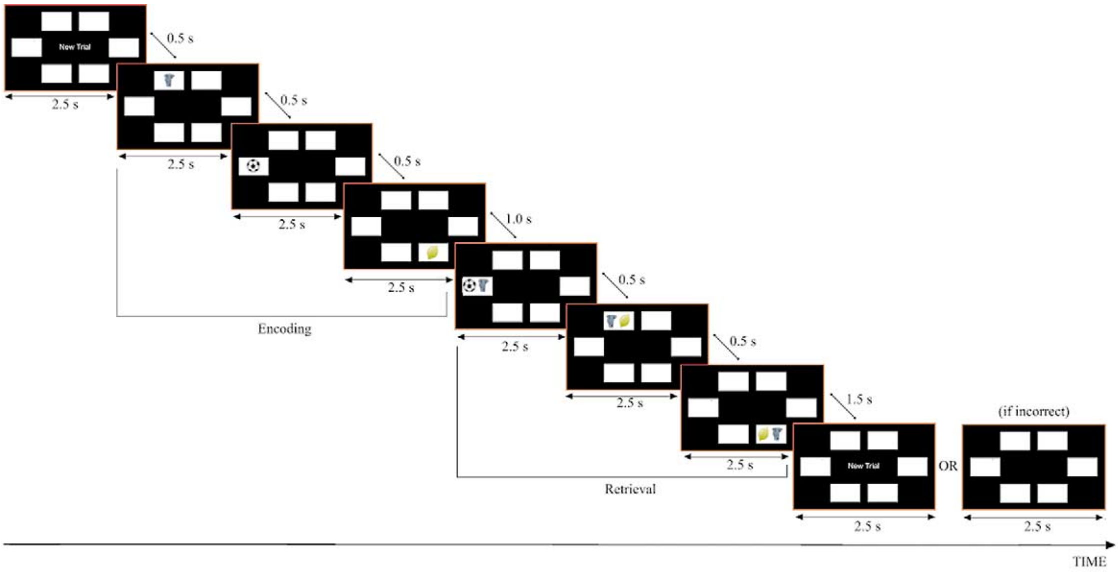


Fig. 1. During encoding three, four, or six objects were individually presented in one of six locations (with each object occupying a different location). Participants were instructed to remember *what* object appeared in *which* location. In the retrieval phase, two previously presented objects appeared side-by-side in one of the six locations. Participants were required to make a forced-choice recognition decision as to which of the two objects previously appeared in that location as a single object. This was repeated until all objects presented in the encoding phase had been responded to. If an incorrect or very slow response was made to any of the object–location decisions then the same objects, together with their locations, were presented in both the encoding and retrieval phases until 100% successful retrieval was achieved (or five failed retrieval attempts had been amassed).

background (located to the left and right top, middle, and bottom of the screen) in which pictures of everyday colour objects (e.g., ant, pencil, violin) were presented (see Fig. 1). In the VERT, participants were required to remember *what* object appeared in *which* location (out of six possible locations) on a computer screen. In the encoding (ENC) phase an object would appear in one of the locations for 2.5 s, followed 500 ms later by the next object in a different location, and so on until three, four, or six different objects had been presented in three, four or six different locations. In the retrieval (RET) phase, occurring 1 s after the end of the ENC phase, two previously presented objects would appear side-by-side in one of the previously used locations for 2.5 s. During this time participants had to make a recognition (left–right) decision as to which of the two objects had appeared in that specific location, by pressing one of two response keys. Five hundred milliseconds after the first recognition decision, two further objects would then

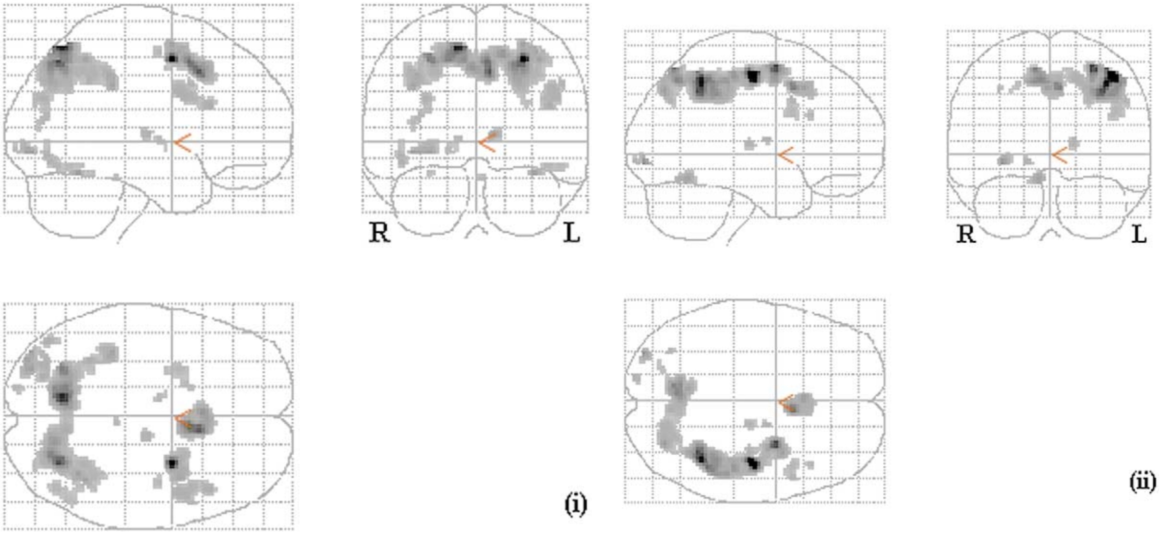


Fig. 2. Regions of significant activation demonstrating a positive zero-order relationship with TD during 100% SA (i) encoding and (ii) retrieval. Only activations with at least five contiguous voxels within a cluster are reported. FDR = 0.0001. Data are presented in radiological orientation (L = R).

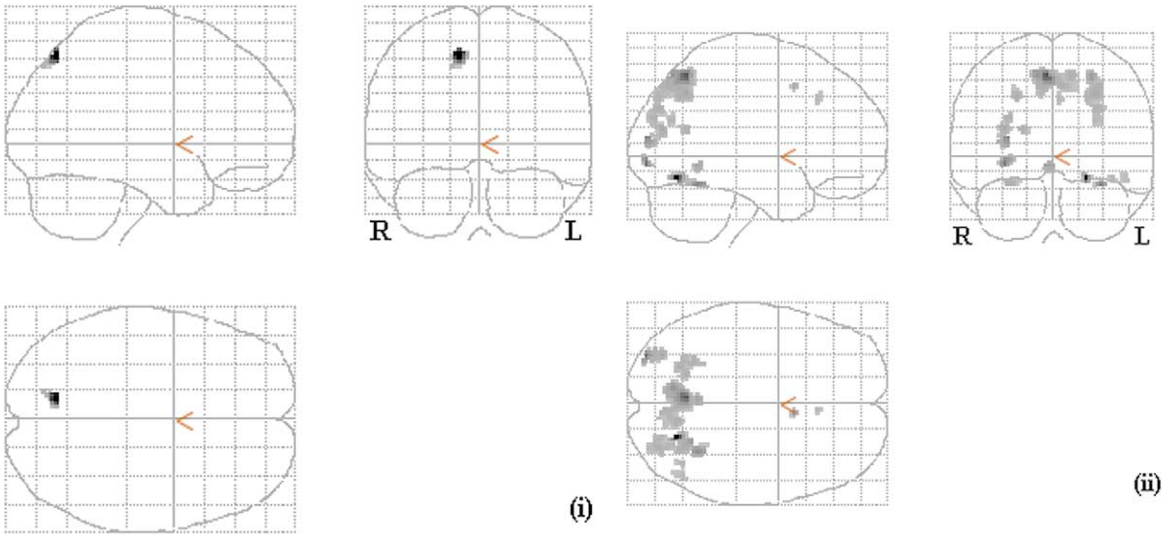


Fig. 3. Regions of significant activation demonstrating a positive linear relationship with TD during (i) encoding (inclusively masked by zero-order encoding) and (ii) retrieval (inclusively masked by zero-order retrieval). First-order and zero-order images were each thresholded at FDR 0.05, with at least five contiguous voxels within each cluster. Data are presented in radiological orientation (L = R).

be presented until all objects seen in the encoding phase had been tested. If an incorrect response was made on any of the object–location decisions then all object–location pairings were repeated in a new encoding phase (4 s after the end of the RET phase) until there was 100% successful retrieval (or until five failed attempts at encoding and retrieval had been amassed). Upon 100% successful retrieval a new set of object–locations would be presented for encoding and retrieval 4 s after the end of the RET phase. The pseudorandom order of object–location problems (e.g., three, four, or six) was counterbalanced across participants, with no more than two problems of the same length appearing consecutively in a scan run. The objects used were counterbalanced across scan runs for each participant with no object being used more than once.

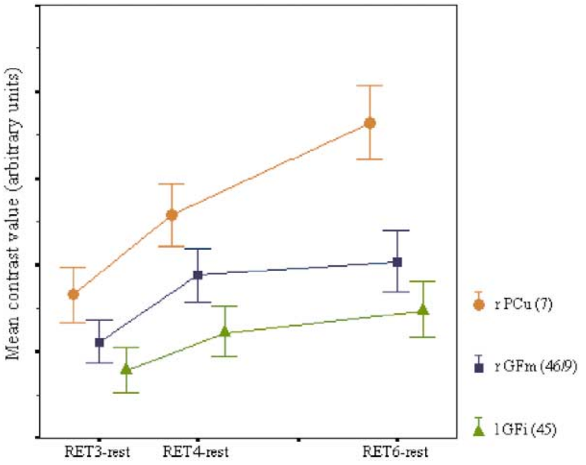


Fig. 5. Mean contrast values for three regions of significant activation identified in a comparison between RET6 vs RET3. Mean contrast values (±standard error of the mean) during retrieval were averaged across all subjects in right precuneus (PCu 2-58 51), right middle frontal gyrus (GFm 44 34 24), and left inferior frontal gyrus (GFi -46 20 17).

The provision of up to five attempts to successfully retrieve object–location pairs in each problem (defined as an entire episode of an encoding and retrieval phase) allowed the examination of encoding and retrieval processes when successful performance had been attained by all subjects (e.g., on the *n*th attempt, hereafter referred to as the 100% successful attempt [100% SA]). This ensures that task performance is controlled across subjects at all levels of task difficulty. It is important to control for performance across subjects because the processes that occur during a failed encoding or retrieval attempt may be very different from those occurring during a successful one.

Prior to online testing, each participant completed two practice sessions: (1) a session occurring 3–4 days before scanning in which individual levels of difficulty were determined based upon the level of successful retrieval reached of trials ranging from two to six object–locations; and (2) a session occurring 1 h before scanning. All participants attained the hardest level of difficulty (memory load of six object–locations). For online testing memory loads of three, four, and six object–locations were chosen, representing easy, medium, and hard levels of difficulty, respectively. After online testing, all subjects were asked to rate how difficult it had been to achieve 100% successful performance at each of the memory loads using a rating scale from 1 = very easy to 7 = very difficult.

Scanning method and image analysis

Functional (T2* weighted) and anatomical (T1 weighted) images were obtained for each participant at the

Wolfson Brain Imaging Centre, Cambridge using a 3T Bruker scanner. Functional data sensitive to the blood oxygenation level dependent signal (BOLD) was acquired using an interleaved echoplanar imaging sequence (TR = 3020 ms, TE = 27 ms, flip angle = 90°, field of view = 25 cm). Each volume consisted of a 128 × 128 matrix and 21 axial slices, with a thickness of 5 mm, a gap of 5 mm, and a voxel size of 1.95 mm × 1.95 mm × 5 mm. The first seven EPI volumes of each run were discarded to allow for magnetic stabilization. Each scanning run lasted a maximum of 8 min or until four problems at each difficulty level had been successfully encoded and retrieved. Two of the participants received only two runs while all other participants received three runs.

All preprocessing of the functional data and subsequent analyses were performed using Statistical Parametric Mapping (Friston et al., 1995) with SPM99 software (Wellcome Department of Cognitive Neurology, London, UK). Due to the acquisition of interleaved EPI images (in which odd slices are collected before even slices) slice timing correction using sinc-interpolation was applied to the data with reference to the first slice of each volume. Within each scan session data were realigned to the first EPI volume of each session, coregistered to the structural T1 weighted image, normalized to Talairach and Tournoux (1988) standard space using nonlinear basis functions and a standard EPI template image based on the Montreal Neurological Institute reference brain (Cocosco et al., 1997), and finally smoothed with an 8 mm full-width Gaussian kernel.

Data analysis

In the parametric analyses, for each subject, the task difficulty parameter (load 3, 4, or 6) was regressed onto the BOLD response to stimuli onsets using an event-related design that was convolved with a canonical haemodynamic response function (HRF) within a general linear model. An event-related rather than the preferred epoch design was specified for the parametric analysis because the modelling of epochs with variable durations within a single regressor precludes parametric modulation within SPM99. Encoding and retrieval trials (with a trial defined as an entire episode of an ENC or RET phase) were treated as separate regressors, with events (or miniepochs) lasting 9, 12, and 18 s for loads 3, 4, and 6, respectively. All unsuccessful encoding and retrieval trials were included as covariates of no interest (one covariate for encoding and one for retrieval). In each model, six movement parameters (three rigid-body translations and three rotations) obtained during image realignment and a constant term were also included as covariates. Global signal intensities were scaled within each session and temporal autocorrelations were adjusted for using a HRF low-pass filter. Linear and nonlinear relationships between memory load and BOLD responses were assessed in a polynomial expansion by first modelling zeroth order (standard boxcar), then first order (linear), and finally second order (quadratic) regressors against a rest baseline (with previous terms being modeled as covariates of no interest upon each new expansion). Load independent, linear load-dependent and nonlinear load-dependent regions were examined by the comparison of BOLD signal changes against a "rest" baseline comprising the 4 s interval between the end of one problem and the beginning of the same problem (if retrieval had been unsuccessful) or another different problem (if retrieval had been successful).

In the categorical analyses, for each subject, the BOLD response to the stimulus onset of each trial type was modelled as a boxcar function and convolved with a canonical haemodynamic response function (HRF) within the general linear model. Encoding and retrieval trials were treated as separate epochs, with epochs lasting 9, 12, and 18 s for loads 3, 4 and 6, respectively. Three encoding and three retrieval epoch-types (each corresponding to three levels of memory load: 3, 4, and 6) were included as covariates, while unsuccessful encoding and retrieval trials were included as covariates of no interest (one per condition-type). In each model a constant term and six movement parameters (three rigid-body translations and three rotations) obtained during image realignment were also included as covariates. Global signal intensities were scaled within each session and temporal autocorrelations were adjusted for using a HRF low-pass filter. Parameter estimates for each covariate were calculated using a least mean squares fit to the data.

For parametric and categorical analyses, SPM{T} contrast images generated from combinations of parameter estimates (e.g., ENC6-ENC3 or RET6-RET3) were produced for each subject (separately for encoding and retrieval) and entered at a second random effects level into one-sample and two-sample t tests, upon which statistical parametric maps (SPMs) of the Z statistic were constructed. All SPMs relating to load contrasts were masked with a load vs rest (or baseline) contrast (e.g., $6 > 3$, masked by $3 >$ rest) to separate differential activations from differential suppressions with respect to the baseline condition.

Unless stated otherwise, all voxel-level contrasts were thresholded at $P < 0.05$, corrected for multiple comparisons using False Discovery Rate (FDR) statistics (Genovese et al., 2002), and only interpreted if at least five contiguous voxels were present within each cluster. As a measure of correcting for multiple comparisons, FDR is based on the *expected proportion* of false positives (or incorrect rejections of the null hypothesis). All EPI images were normalised using a standard EPI template image based on the Montreal Neurological Institute reference brain (which does not perfectly match the Talairach and Tournoux brain) and so coordinates provided by SPM were converted from MNI space to Talairach and Tournoux (1998) space (Brett et al., 2001).

Table 1
Behavioural measures of performance on an object–location paired associate task

Task diff. level	Mean % correct on 1st RA		Mean no. attempts		Mean rating of task diff.	
	Mean	SE	Mean	SE	Mean	SE
3	0.52	0.07	1.30	0.05	1.91	0.19
4	0.44	0.09	1.34	0.06	3.00	0.28
6	0.15	0.10	1.61	0.10	4.86	0.31

Note. For rating of task difficulty, $n = 21$ (nine young and 12 old subjects); for other analyses $n = 23$ (11 young and 12 old subjects). Values for mean % correct on 1st RA are scaled so that 50% = 0 and 100% = 1 (because 50% accuracy would be predicted by chance in a two-response forced choice task).

Results

Behavioural data analysis

For each condition, the percentage of problems correct on the first retrieval attempt (first RA, scaled so that 50% = 0 and 100% = 1 because 50% accuracy would be predicted by chance in a two-response forced choice task), the number of attempts taken to successfully complete a problem and the subjective rating of difficulty were obtained for each subject. The behavioural data are shown in Table 1.

Data were submitted to one-factor related analyses of variance (ANOVA), with the within-subject variable of task difficulty (three, four, or six object–location pairs). For the mean percentage of problems correct on the first RA, the main effect of TD was significant, $F(2, 44) = 14.15$, $MSE = 0.86$, $P < .0001$. Post hoc analyses using Tukey pairwise comparisons revealed that fewer six pair trials were correctly completed on the first RA compared to three or four pair trials, while there was no difference between three and four pair trials (3 vs 4, $q = 1.62$, n.s.; 3 vs 6, $q = 7.17$, $P < 0.01$; 4 vs 6, $q = 5.55$, $P < 0.01$).

For the mean number of attempts to successfully complete a problem the main effect of TD was significant, $F(2, 44) = 13.07$, $MSE = 0.65$, $P < .0001$. Post hoc Tukey pairwise comparisons revealed that more attempts were taken to achieve 100% successful retrieval of six pair trials compared to three or four pair trials, with no difference between three and four pair trials (3 vs 4, $q = 0.84$, n.s.; 3 vs 6, $q = 6.64$, $P < 0.01$; 4 vs 6, $q = 5.80$, $P < 0.01$).

Finally, for the subjective ratings of difficulty, the main effect of TD was significant, $F(2, 40) = 50.42$, $MSE = 46.78$, $P < .0001$. Post hoc Tukey pairwise comparisons revealed that six pair trials were rated as being subjectively more difficult than three or four pair trials, and four pair trials were rated as being subjectively more difficult than three pair trials (three vs four $q = 5.21$, $P < 0.01$; 3 vs 6, $q = 14.05$, $P < 0.01$; 4 vs 6, $q = 8.84$, $P < 0.01$).

To summarize, fewer trials were successfully completed on the first RA, more attempts were taken to achieve 100% successful retrieval, and subjective ratings of difficulty were greater for six object–location pair trials compared to three or four pair trials. No performance differences were observed between three and four pair trials despite four pair trials being rated as subjectively more difficult than three pair trials.

fMRI data analysis: encoding and retrieval

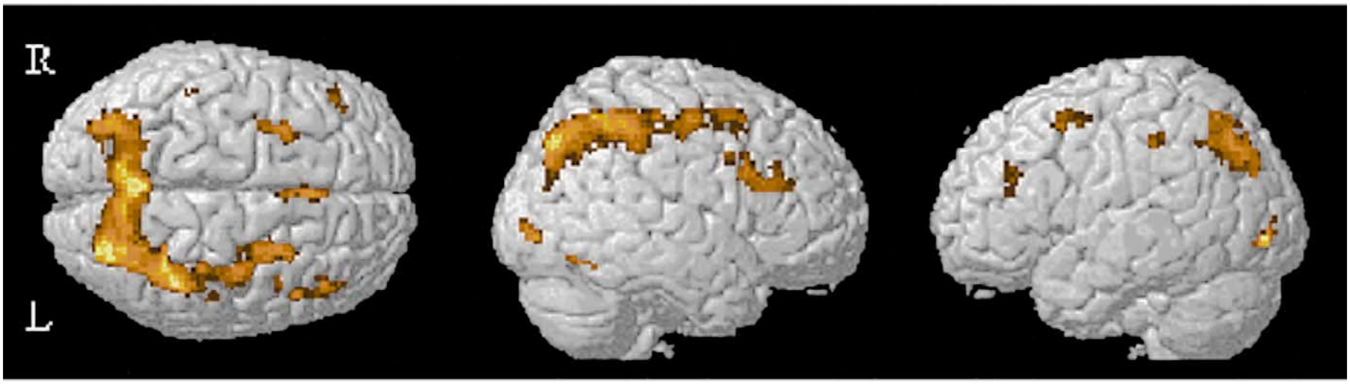
For both encoding and retrieval comparisons, 100% SA (100% successful attempt) corresponds to the attempt at which the problem is successfully completed (for encoding this is data associated with subsequent 100% successful retrieval). A two-sample t test (young vs old subjects) conducted on parametric contrasts (e.g., zero order vs baseline, first order vs baseline, second order vs baseline) and categorical contrasts (e.g., 6 minus 3, 6 minus 4, 4 minus 3) revealed a lack of statistically significant age-related differences on the 100% SA. Accordingly, young and old data were pooled for all subsequent analyses at the random effects level, with between-subject comparisons being made using one sample t tests ($n = 23$). Due to numerous difficulties in the interpretation of deactivations or suppressions only activations are reported here.

Parametric analyses: boxcar (zero order) function for encoding and retrieval

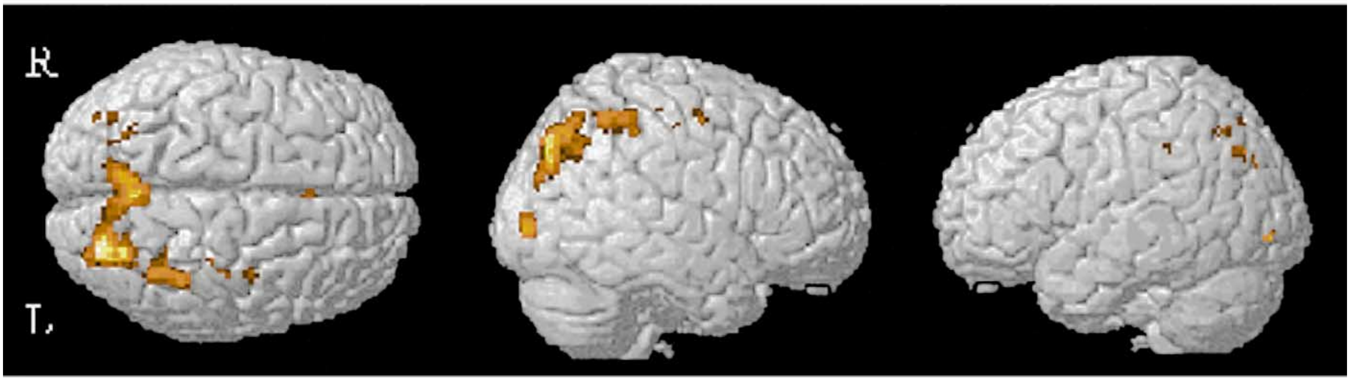
We began with identification of the regions displaying a positive zero-order relationship between memory load and BOLD responses on the attempt associated with 100% successful retrieval during encoding and retrieval. Zero-order terms can be modelled using a categorical on–off boxcar function that sums over memory load conditions (with respect to a baseline condition). The results for the comparison between the zero-order regressor and the "rest" baseline for encoding and retrieval are shown in Table 2 and Fig. 2.

When the relationship between memory load and BOLD signal changes was characterized using a boxcar function, similar patterns of activation during encoding and retrieval of object–location pairs were identified. Irrespective of memory load, compared to a "rest" baseline signal, intensity changes were found in multiple occipital–parietal, cerebellum, inferior and middle frontal gyri, and anterior cingulate regions during both encoding and retrieval. In contrast to other parametric and categorical analyses, activations in the zero-order parametric analysis were thresholded at FDR 0.0001 because large clusters of activation (19,000 voxels occurring in one cluster during encoding and 11,000 voxels occurring in one cluster during retrieval) were found at FDR 0.05. There are two reasons why a more stringent statistical threshold was chosen for the zero-order analysis compared to other analyses. First, such large clusters are difficult to interpret given that many different brain regions may be encompassed within a single cluster. Second, a feature of the false discovery rate is that the FDR thresholds are adaptive (Genovese et al., 2002); as signal extent increases,

(i) RET6 > RET3 (masked by RET3 > rest)



(ii) RET6 > RET4 (masked by RET4 > rest)



(iii) RET4 > RET3 (masked by RET3 > rest)

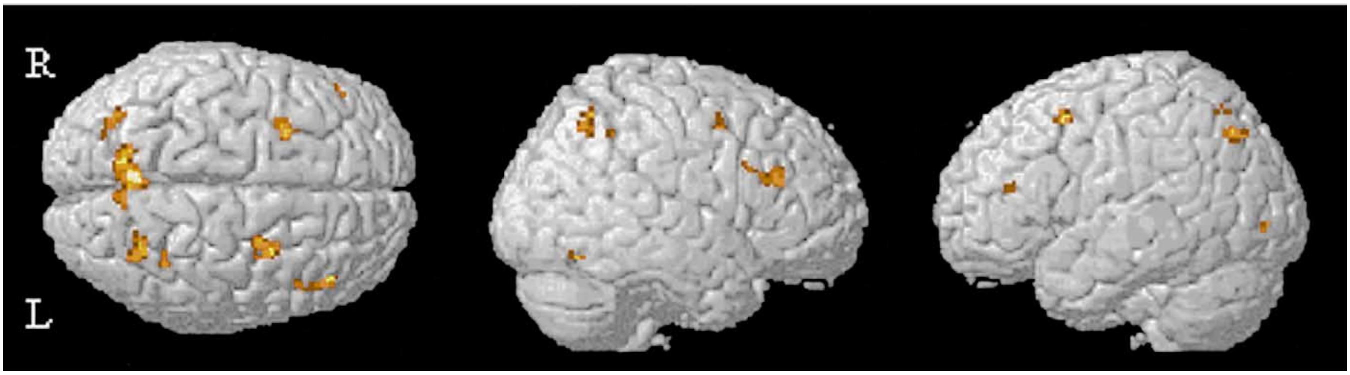


Fig. 4. Surface renderings of regions of significant signal change in comparisons between levels of retrieval. Only activations with at least five contiguous voxels within a cluster are reported. FDR = 0.05. Data are presented in radiological orientation (L = R).

the FDR threshold decreases. Because FDR is based upon the proportion of activated voxels, as signal extent increases so too can the number of expected false discoveries (c.f. Nichols, 2002). If this number is large then the FDR *q* threshold can be altered until a more reasonable expected number of false positives is achieved. For example, in a zero-order parametric analysis of encoding data, and FDR *q* value of 0.05 yields 1094 expected false discoveries, while an FDR value of 0.0001 yields a more acceptable 0.30 expected false discoveries.

When regions exhibiting a zero-order relationship with TD were compared across encoding and retrieval (in a one-sample *t* test), greater signal intensity changes were found in regions including the inferior lateral frontal, post-

Table 2
Regions of significant activation demonstrating a positive zero-order relationship with TD during 100% SA (i) encoding and (ii) retrieval

Region	Hemisphere/BA	*Z* score	*x*	*y*	*z*
(i) Zero order encoding					
Middle frontal gyrus	L6	6.70	−28	2	46
Precuneus	R7	6.45	12	−61	55
Anterior cingulate gyrus	L32	6.35	−8	17	38
Cerebellum	L	5.72	−34	−56	−18
Inferior occipital gyrus	R18	5.70	28	−82	−1
Inferior frontal gyrus	L45	5.44	−44	22	19
Precuneus	R19	5.30	24	−74	42
Middle occipital gyrus	R19	5.29	46	−72	−3
Superior occipital gyrus	R19	5.27	30	−74	28
Thalamus	L	5.20	−12	−13	4
Precentral gyrus	L4	5.07	−40	−5	50
Middle frontal gyrus	R6	5.04	28	6	46
Cerebellum	R	4.89	28	−47	−13
Superior occipital gyrus	L19	4.87	−30	−78	26
(ii) Zero order retrieval					
Precentral gyrus	L4	6.40	−42	−13	49
Anterior cingulate gyrus	L32	5.88	−6	14	40
Inferior occipital gyrus	R18	5.66	32	−88	−4
Cerebellum	R	5.52	8	−59	−11
Thalamus	L	5.19	−16	−17	8
Inferior frontal gyrus	L44	5.12	−50	11	33
Precuneus	R19	4.96	34	−66	42
Precuneus	R7	4.87	22	−72	46

Note. Only activations with at least five contiguous voxels within a cluster are reported. FDR = 0.0001. BA = Brodmann's area. All coordinates (in mm) have been converted from MNI space to Talairach and Tournoux space. During encoding, a large cluster (1809 voxels) in the right-sided precuneus included regions of significant activation in bilateral superior parietal lobule (BA 7), left precuneus (BA 7), and bilateral inferior parietal lobule (BA 40). During retrieval, a large cluster (1785 voxels) in the left-sided precentral gyrus included regions of significant activation in left inferior parietal lobule (BA 40), bilateral precuneus (BA 7), left superior parietal lobule (BA 7), and left middle frontal gyrus (BA 6).

central gyrus, and medial temporal (right hippocampus and left parahippocampal gyrus) cortices during encoding compared to retrieval and in the inferior parietal lobule, precentral gyrus, and cerebellum during retrieval compared to encoding (see Table 3).

Linear (first order) and nonlinear (quadratic) terms for encoding and retrieval

Following fitting of the model with a zero-order term, a first order term was included in order to assess the linearity of the relationship between memory load and BOLD responses on the attempt associated with 100% successful retrieval during encoding and retrieval. By including an additional linear term we are able to determine regions in which the relationship between signal change and memory load can be better characterized (in terms of accounting for the variance) by a first order term rather than zero-order term. When examining positive linear relationships between TD and BOLD responses, an inclusive mask of the zero-order term was used to ensure that any resulting activations were greater than the baseline condition. The results for the comparison between the first order term and baseline condition for encoding and retrieval are shown in Table 4 and Fig. 3.

During the encoding attempt associated with 100% successful retrieval, a significant positive linear relationship with memory load (as load increased, signal change increased) was found in the right-sided precuneus. During retrieval, the addition of a positive linear term accounted for variance in the middle occipital gyrus bilaterally, left superior occipital gyrus, left fusiform gyrus, bilateral cerebellum, right precuneus, and anterior cingulate gyrus. There were no statistically significant differences between regions displaying a positive linear relationship with load during encoding compared to retrieval (and vice versa).

For both encoding and retrieval, the inclusion of a nonlinear quadratic (second order) term in the parametric model did not better characterize the relationship between load and signal change in regions fitted by zero-or first-order terms. However, the ability to detect nonlinear increases in signal change with increasing memory load is likely to have been compromised by an insufficient number of data points (three levels of memory load).

Summary of parametric analyses

During retrieval associated with the 100% successful attempt, multiple occipital–parietal regions, cerebellum, and

Table 3
Regions of significant activation in the (i) encoding vs retrieval positive zero-order conditions and (ii) retrieval vs encoding positive zero-order conditions

Region	Hemisphere/BA	*Z* score	*x*	*y*	*z*
(i) ENC minus RET					
Inferior frontal gyrus	L47	5.78	−36	26	−18
Middle temporal gyrus	R39	5.31	40	−52	10
Middle frontal gyrus	R11	5.29	28	34	−17
Medial frontal gyrus	L11	5.28	−4	54	−14
Inferior frontal gyrus	L47	5.26	−48	25	−6
Hippocampus	R	5.13	28	−12	−11
Postcentral gyrus	L4	5.11	−57	−5	22
Cuneus	R18	4.95	16	−84	23
Postcentral gyrus	R4	4.94	52	−5	17
Parahippocampal gyrus	L36	4.78	−30	−34	−13
(ii) RET minus ENC					
Cerebellum	R	6.49	6	−63	−10
Inferior parietal lobule	L40	5.88	−38	−48	45
Precentral gyrus	L4	5.87	−38	−15	52

Note. Only activations with at least five contiguous voxels within a cluster are reported. FDR = 0.0005. BA = Brodmann's area. All coordinates (in mm) have been converted from MNI space to Talairach and Tournoux space.

Table 4
Regions of significant activation demonstrating (i) a positive linear relationship with TD during 100% SA encoding (inclusively masked by zero-order encoding) and (ii) a positive linear relationship with TD during 100% SA retrieval (inclusively masked by zero-order retrieval)

Region	Hemisphere/BA	Z score	x	y	z
(i) First-order encoding					
Precuneus	R7	5.07	12	−65	51
(ii) First-order retrieval					
Fusiform gyrus	L19	5.33	−22	−66	−8
Precuneus	R7	4.93	4	−58	53
Middle occipital gyrus	R18	4.71	32	−85	1
Cerebellum	L	4.55	−32	−53	−12
Middle occipital gyrus	R19	4.48	32	−83	15
Anterior cingulate gyrus	L32	3.99	−6	12	42
Cerebellum	R	3.97	28	−55	−12
Superior occipital gyrus	L19	3.95	−30	−76	33
Middle occipital gyrus	L19	3.68	−40	−63	−10
Anterior cingulate gyrus	L32	3.62	−4	27	34
Middle occipital gyrus	L19	3.48	−46	−66	−7

Note. First-order and zero-order images were each thresholded at FDR 0.05, with at least 10 contiguous voxels within each cluster. BA, Brodmann's area. All coordinates (in mm) have been converted from MNI space to Talairach and Tournoux space.

anterior cingulate gyrus displayed a positive linear relationship with load. During the encoding attempt associated with 100% successful retrieval, similar occipital–parietal, cerebellum, and anterior cingulate regions were better described by a boxcar (zero-order) function, with the right precuneus being the only region to exhibit significant linear load dependency. Significant differences between encoding and retrieval (in regions exhibiting a zero-order relationship with load) were found in inferior lateral frontal and medial temporal cortices during encoding (compared to retrieval) and in the inferior parietal lobule and cerebellum during retrieval (compared to encoding).

Nonlinear load-dependent responses were not identified during encoding or retrieval associated with the 100% successful attempt, although Callicott et al. (1999) have reported three types of load-sensitive activations within frontoparietal regions in a verbal *n*-back WM study. A plateau response was found in the right precuneus, an inverted-U response was found in the left DLPFC, and a linear response was found in the medial pericingulate as load increased. Because the detection of nonlinear effects in the current study may in part be due to the insufficient number of memory load conditions, we were interested in exploring these possible load-sensitive activations further. In the following analyses, subtractions between individual levels of memory load (three, four, or six object–location pairs) were conducted in the hope that they would identify areas displaying nonlinear trends (e.g., activations that are significant in some contrasts but not others).

Categorical analyses: encoding and retrieval task difficulty contrasts

In comparisons between ENC6/ENC4 and ENC4/ENC3, no significant activation differences were found following correction for multiple comparisons (i.e., at a corrected height threshold), while significant signal intensity changes were found in all comparisons between levels of retrieval. Accordingly, nonlinearity could only be assessed during the retrieval attempt associated with 100% successful performance. Two regions that appeared to exhibit a nonlinear plateau response to increasing task load were the right dorsolateral prefrontal cortex (DLPFC: 44 34 24 [BA 46] in RET 6 vs RET 3 and 50 34 13 [inferior part of BA 46, at the boundary with BA 45 and close to the inferior frontal sulcus] in RET 4 vs RET 3) and left inferior frontal gyrus (IFG: −46 20 17 [BA 44] in RET 6 vs RET 3 and −48 17 25 [BA 44] in RET 4 vs RET 3) in which significant activation changes were found in contrasts between RET6 vs RET3 and RET4 vs RET3 but not RET6 vs RET4 (see Fig. 4). These regions were not previously identified in the zero-order parametric analysis, however, this may simply be a consequence of thresholding at FDR 0.0001 and so may represent signal changes that were just below the statistical significance threshold. There were no regions that appeared to display an inverted U response.

Responses in the right DLPFC, left IFG, and right precuneus during retrieval were further examined in each subject by determining mean contrast values across load conditions (relative to a rest condition) for significant peak activations identified in a comparison between RET6 vs RET3. Signal intensity values were then averaged across all subjects to produce the load–response graph displayed in Fig. 5. Mean contrast values for each region were submitted to separate one-factor related ANOVAs, with the within-subject variable of task difficulty (load 3, 4, or 6). In all analyses the main effects of TD were significant: precuneus, F(2, 44) = 45.3, MSE = 5.7, $P < .0001$; DLPFC, F(2, 44) = 13.9, MSE = 1.4, $P < .0001$; IFG, F(2, 44) = 9.0, MSE = 0.7, $P < .001$. Post hoc analyses using Tukey pairwise comparisons revealed significantly greater contrast values for load 6 trials compared to load 4 trials and for load 4 trials compared to load 3 trials in the precuneus (3 vs 4, $q = 6.18$; 3 vs 6, $q = 13.45$; 4 vs 6, $q = 7.27$; all significant at $P < 0.01$). However, in the DLPFC and IFG, significantly greater contrast values were found for comparisons between loads 3 and 4 (DLPFC: 3 vs 4, $q = 5.77$, $P < 0.01$; IFG: 3 vs 4, $q = 3.77$, $P < 0.05$) and between loads 3 and 6 (DLPFC: 3 vs 6, $q = 6.96$, $P < 0.01$; IFG: 3 vs 6, $q = 5.92$, $P < 0.01$), but not between loads 4 and 6 (DLPFC: 4 vs 6, $q = 1.19$, n.s.; IFG: 4 vs 6, $q = 2.15$, n.s.). From this load–response graph, a nonlinearity interpretation is supported because the mean contrast values (obtained by comparing load conditions with a baseline condition) for the right precuneus increased linearly with load, while the contrast values for the right DLPFC and left IFG resembled a plateau function.

Discussion

As memory load increased during the encoding or retrieval attempt associated with 100% successful performance, we observed signal intensity changes in anterior cingulate, and parietal and occipital areas that displayed load independency during encoding (apart from the precuneus for which signal change increased linearly with load) but linear load dependency during retrieval. The addition of a nonlinear (quadratic) regressor into the parametric model did not better describe the BOLD responses during encoding or retrieval. Parametric analyses, however, may have been unable to clearly differentiate nonlinearity due to insufficient data points and so categorical analyses were conducted. Contrasts between levels of TD for retrieval revealed areas in the right DLPFC and left inferior frontal gyrus that exhibited a nonlinear (plateau function) relationship with increasing load. The behavioural data indicated that TD increased as memory load increased, with the percentage of problems correct on the first attempt decreasing and the mean number of attempts taken to successfully complete a problem increasing as load increased from three or four object–locations to six object–locations.

Load independency during encoding but linear load dependency during retrieval in the same network of occipitoparietal and cingulate regions may be a product of the cognitive demands (e.g., attentional processing) required to complete the encoding or retrieval tasks. During encoding, no overt or explicit responses are required and even though subjects are instructed to memorise object–location information, processing is less demanding (and less active) compared to that during retrieval. Such nondemanding processing would be particularly expected on the encoding attempt associated with 100% successful retrieval (compared to previous unsuccessful attempts), during which minimal learning is likely to be occurring, with processing serving either to consolidate previously presented items or to successfully encode 1 or 2 previously incorrect items. Additionally, on the encoding attempt associated with 100% successful retrieval few differences between encoding of three vs four vs six object–location pairs may be evident as a function of minimal paired-associate learning requirements, and so a zero-order rather than linear relationship between signal change and load would be expected. During retrieval, however, processing is more demanding (and more active) compared to encoding, with overt or explicit responses (forced-choice recognition decision) being required subsequent to information retrieval. On the attempt associated with 100% successful performance, regardless of whether information has been learned, retrieval is still prone to interference between items (with interference increasing as the number of items increase) and so effects of TD would be expected to increase in a linear (or nonlinear) fashion. Consequently, we would tentatively suggest that load responses may be modulated either by the level of processing demands or by an interaction between successful performance and the level of processing demands.

Previous studies examining the effects of TD in WM have reported bilateral signal increases in frontoparietal cortices with increasing memory load (Braver et al., 1997; Carlson et al., 1998; Diwadkar et al., 2000; Jonides et al., 1997; Klingberg et al., 1997; Rypma et al., 1999). Specifically, in these studies, bilateral middle and inferior frontal gyri, posterior parietal cortex, and anterior cingulate activations have been associated with increasing TD. In the present study a similar network of brain regions was found to respond to increasing levels of cognitive load, particularly during retrieval of object–location information. For all participants, as memory load increased, signal intensity changes within these areas either remained constant (in encoding) or increased (in retrieval) during 100% successful performance, regardless of the number of attempts taken to learn *what* object appeared *where*. While other studies have demonstrated the recruitment of additional brain regions or a combination of increasing activation in the same regions together with additional regions as TD increased (e.g., Klingberg et al., 1997; Rypma et al., 1999), the current study appeared to show increases in activation in the same, rather than additional, brain areas during retrieval.

By ensuring that performance during encoding and retrieval processing was matched across participants we were able to determine the brain regions that are associated with increasing TD while not being confounded by the failing performance that usually accompanies increasing TD. Other studies have examined the effect of increasing cognitive load in WM, either by manipulating the number of trials to be monitored and updated (e.g., in an *n*-back task), the number of items to be maintained, or the time period over which items should be maintained (Braver et al., 1997; Rypma et al., 1999; Smith et al., 1995). However, few studies have manipulated TD during paired associate learning of object and spatial information. The finding of regions responsive to load in a paired associate learning task, similar to those found in WM tasks that have varied TD (and, indeed, in other tasks that have varied cognitive demands), serves to corroborate the task-independent network of activation (involving frontoparietal cortices) thought to subserve increasing cognitive demands.

The relationship between TD and BOLD signal change

In previous studies, a monotonic relationship between memory load and BOLD fMRI response in prefrontal and parietal cortices has been reported (Braver et al., 1997; Carlson et al., 1998; Cohen et al., 1997; Kammer et al., 1997). However, data reported by Callicott et al. (1999) suggest that areas of activation associated with a given cognitive task may individually vary in their response to increasing load. Callicott et al. (1999) found load-sensitive activations in multiple areas including bilateral inferior, middle and superior frontal gyri, right inferior and left superior parietal lobule, right precuneus, and right medial pericingulate during a verbal *n*-back WM task. "Capacity constrained" or "inverted-U response" (signal change increases with load, peaks at maximum capacity, and then decreases), "capacity independent" (signal change increases initially with load and then reaches a plateau), and "capacity unconstrained" (signal change linearly increases with load) relationships were exhibited in the left DLPFC, right precuneus, and medial pericingulate, respectively.

Our data support previous reports of monotonic increases in BOLD response with increasing cognitive load. Load-dependent or "capacity unconstrained" activations in the occipitoparietal, cerebellum and anterior cingulate regions were found during retrieval, while signal change in the precuneus alone increased linearly with load during encoding (other occipitoparietal, cerebellar and cingulate regions demonstrated load independency). Of course, because nonlinearity may not have been adequately assessed in the parametric analyses, some of these linear increases in activation during retrieval processing may actually represent a "capacity constrained" (inverted-U) response, with signal change increasing initially with lower memory load and then decreasing with higher memory loads. Similarly, signal increases within the right DLPFC and left inferior frontal gyrus (found in the contrasts between RET6 vs RET3 and RET4 vs RET3 but not RET6 vs RET4) may represent a "capacity constrained" (inverted-U response) relationship between load and signal change. However, based on mean contrast values (see Fig. 5), BOLD response within these regions increased initially with load and then reached a plateau (a "capacity independent" response).

Frontal cortical responses to increasing task difficulty

One difference in the present results compared to those of previous task difficulty studies is the lack of bilateral activation in frontal cortical areas in parametric and categorical analyses. While others have reported bilateral changes in middle and/or inferior frontal gyri as TD increased (e.g., Carlson et al., 1998; Jonides et al., 1997; Klingberg et al., 1997; Rypma et al., 1999) the current study demonstrated monotonic signal intensity changes but only in the same unilateral frontal regions (right DLPFC and left inferior frontal gyri) during retrieval processing in categorical analyses. The unilateral activation we have found may simply be an outcome of only examining successful performance during paired associate learning, with frontal activations lateralising in response to task-specific demands or failure.

Left inferior frontal (BA 44/45) and right middle frontal (BA 46, 9) gyri activations have been reported in a face (or object) WM task compared to a control task (Courtney et al., 1996) and in a verbal WM task (Braver et al., 1997; Rypma et al., 1999). Signal changes in the right middle frontal gyrus have been postulated to underlie the executive components of WM (Rypma et al., 1999), while those in the left inferior frontal gyrus (BA 44) are thought to reflect rehearsal processes (Paulesu et al., 1993). Hence, unilateral frontal activations found with categorical contrasts in the current study are consistent with previous observations are coherent with the cognitive functions thought to underlie these activations.

Functionality of regions associated with increasing TD

Whether frontoparietal and occipital activations associated with TD during encoding and retrieval of paired associates were attributable to increasing mental effort or arousal or to mnemonic processes could not be ascertained in the current study. Duncan and Owen (2000) concluded that activations in mid-DLPFC, mid-VLPFC, and anterior cingulate were typically found under conditions of increasing cognitive demand, irrespective of the type of task (mnemonic vs nonmnemonic). This suggests that lateral and medial frontal activations in the current study evinced from parametric analyses and categorical comparisons between different levels of memory load may be related to TD rather than WM load. However, Barch et al. (1997) reported left inferior and middle frontal gyrus activity associated with mnemonic factors (while controlling for TD) and right inferior frontal gyrus activity associated with task difficulty (while controlling for WM load) in a continuous performance WM task. Certainly, increases in signal change in left and right middle frontal and inferior frontal gyri have been reported in studies of WM. Activation of the VLPFC is posited to underlie either nonspatial processing or maintenance of information in WM, and DLPFC activations are thought to reflect spatial processing, manipulation of information within WM, or the central executive component of WM (Courtney et al., 1997; Goldman-Rakic, 1996; Owen et al., 1996a; Petrides, 1996). Rypma et al. (1999) have also suggested that DLPFC may be activated when the maintenance capacity of information in WM is exceeded (hence engaging central executive processes), with ventral regions being activated during subcapacity maintenance. Thus, further investigation is required to determine the exact role of load–responsive frontoparietal regions engaged in the present study.

With respect to posterior parietal and occipital cortices, activation in these regions has been reported in visuospatial WM tasks (Belger et al., 1998; Jonides et al., 1993), with greater activation in the occipitotemporal or ventral visual pathway being found during object WM (compared to spatial WM), and greater activation in the occipitoparietal or dorsal visual pathway being found during spatial WM (compared to object WM) (Courtney et al., 1996; Owen et al., 1996b). Further, left posterior parietal cortex showed greater activation in a long compared to short delay condition (while controlling for TD) and so was considered to be related to mnemonic rather than TD factors (Barch et al., 1997). In terms of cognitive function, activations in parietal regions have been attributed to verbal information storage processes in WM (Awh et al., 1996; Paulesu et al., 1993; Smith et al., 1996) or to nonmnemonic processes such as spatial perception (Ungerleider and Mishkin, 1982), spatial computation (Carpenter *et al.*, 1999), and the engaging or disengaging of spatial attention (Corbetta et al., 1998; Posner and Petersen, 1990). Activity in the occipital cortex, though common in WM tasks, has been related to nonmnemonic processes of visuospatial perception or recognition (Ungerleider and Mishkin, 1982).

Finally, the association between anterior cingulate activity and TD (rather than mnemonic factors) has been well documented. Activation of the anterior cingulate was found under conditions of visual degradation when controlling for WM, but not when varying delay in WM while controlling for TD (Barch et al., 1997). Across a range of mnemonic and nonmnemonic tasks, signal increase in the anterior cingulate has been found under conditions of increased cognitive load (Duncan and Owen, 2000), while Paus et al. (1998) concluded that increased arousal during the performance of difficult or attentionally demanding tasks, together with WM demands, mediates the effects of TD on blood flow in the supracallosal anterior cingulate. In functional terms, the anterior cingulate has been related to initiation or willed control of behaviour (Paus, 2001; Posner et al., 1988) and inhibition of inappropriate responses (George et al., 1994). Hence, while the contribution of TD but not mnemonic factors to anterior cingulate and occipitoparietal activation is relatively clear, frontal activations are less dissociable in a mnemonic task that varies task difficulty, within which both factors are likely to underlie resulting activations.

Lack of ageing effects in fMRI data

Due to our examination of the influence of TD upon encoding and retrieval when performance was 100% successful (i.e., when performance was matched across groups), we found significant behavioural differences between older and younger adults in the current study. We were not interested in these behavioural differences because they were an inevitable by-product of analysing the 100% SA. However, it is useful to note that older adults successfully retrieved fewer object–location pairs on the first RA and took more attempts to achieve 100% successful retrieval than younger adults (especially when six object–location pairs had to be encoded or retrieved).[1,2]

In the current study, we found no significant differences in functional activation between older and younger adults, in contrast to numerous other studies that have reported such ageing effects (c.f. Cabeza, 2001 for a review of ageing effects in neuroimaging studies). This result may seem disappointing. However, it is of noteworthy interest because it demonstrates that despite taking more attempts to reach the same level of performance as younger adults, older adults use the same network of brain regions to respond to increasing levels of task difficulty as younger adults.

The lack of between-group differences in analyses of fMRI data is likely due to our choice of analysing the 100% SA rather than the first RA. If we were to compare older vs younger adults on the trials when only one attempt was taken to achieve 100% successful performance then we might start to see significant differences in functional activation between the two groups. This is difficult to assess in the current study given that few older participants managed to respond correctly on the first attempt, particularly in the higher load conditions. Furthermore, interpretation of these differences would be clouded by the fact that task difficulty would most likely differ between groups (because responding correctly on the first attempt would probably be harder for the older adults than younger adults, especially as load increased).

In terms of physiological or statistical factors that may influence the ability to detect significant differences in BOLD response between older and younger groups, there are two factors that may be masking between-group differences. First, an increased spatial variability in the BOLD signal in the older group, and second, a large intersubject variability in the magnitude of the BOLD response within the older group. Buckner et al. (2000) have demonstrated significant differences in the amplitude of the BOLD re-

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[1] Data were submitted to two-factor mixed analyses of variance (ANOVA), with the within-subject variable of task difficulty (three, four, or six, object–location pairs) and the between-subject variable of age (young or old adults). For the mean percentage of problems correct on first RA the main effects of age and TD and the age by task difficulty interaction were significant: F(1, 42) = 25.94, MSE = 4.76, $P < .0001$ (0.65 vs 0.12 for younger vs older group); F(2, 42) = 15.72, MSE = 0.83, $P < .0001$ (3 = 0.52, 4 = 0.44, 6 = 0.15); F(2, 42) = 4.47, MSE = 0.23, $P < .05$. Post hoc analyses using Tukey pairwise comparisons revealed that older subjects successfully retrieved fewer object–location pairs on the first RA than younger subjects across all levels of task difficulty (3, $q = 3.30$, $P < .05$; 4, $q = 6.34$, $P < .01$; 6, $q = 7.59$, $P < .01$). For younger subjects, there were no differences in the number of problems correct on the first RA across levels of TD (3 vs 4, $q = 0.90$; 3 vs 6, $q = 2.37$; 4 vs 6, $q = 3.26$). For older subjects, fewer load 6 problems were correctly completed on the first RA compared to load 3 and 4 problems (3 vs 4, $q = 3.28$; 3 vs 6, $q = 8.42$, $P < 0.01$; 4 vs 6, $q = 5.14$, $P < 0.01$).

[2] For the mean number of attempts to successfully complete a problem the main effects of age and TD and the age by task difficulty interaction were significant: F(1, 42) = 19.11, MSE = 2.73, $P < .0001$ (1.20 vs 1.60 for younger vs older group); F(2, 42) = 14.91, MSE = 0.57, $P < .005$ (3 = 1.30, 4 = 1.34, 6 = 1.60); F(2, 42) = 6.53, MSE = 0.25, $P < .05$. Pairwise comparisons revealed that the mean number of attempts was greater for older than younger subjects at loads 4 and 6, but not load 3 (3, $q = 2.43$; 4, $q = 4.93$, $P < 0.01$; 6, $q = 7.63$, $P < 0.01$). For younger subjects there was no difference in the mean number of attempts across levels of TD (3 vs 4, $q = 1.18$; 3 vs 6, $q = 1.33$; 4 vs 6, $q = 2.51$). For older subjects, the mean number of attempts was greater for load 6 problems compared to load 3 and 4 problems (3 vs 4, $q = 2.33$; 3 vs 6, $q = 8.78$, $P < 0.01$; 4 vs 6, $q = 6.45$, $P < 0.01$).

sponse between younger and older (demented and nondemented) adults in the visual cortex (though similar amplitudes across groups within the motor cortex), while D'Esposito et al. (1999) reported significantly greater voxel-averaged noise in motor haemodynamic responses in older compared to younger adults. Given these observed differences in the properties of the BOLD response between younger and older adults, together with the analysis of the 100% SA, the lack of statistically significant age differences in functional activation in the current study is not altogether surprising.

Number of attempts and memory load

The matching of performance across age groups, though ensuring that regions responding to increasing TD were not confounded by failing performance, raises the possibility that some activations attributed to increasing memory load may in fact represent an increase in the number of trial attempts. Because signal change decreases are generally found as trials are repeated it is unlikely, however, that this is the case. Decreased activity due to the repeated presentation of a trial is commonly reported in studies of repetition priming and learning (e.g., Schacter and Buckner, 1998). For example, in a study examining repetition priming of object recognition, decreases in BOLD response were found in occipitotemporal and posterior parietal regions (e.g., James et al., 1999). Within the learning domain, signal change decreases or repetition suppression effects associated with the (encoding or) learning of novel object–location pairings across eight trials have been found in the primary visual cortex, inferotemporal, dorsal extrastriate, and posterior parietal cortices (Büchel et al., 1999). Because signal change increases in occipital and posterior parietal regions have been reported in the current study as memory load increased (with care taken to ensure that differential activations were separated from differential suppressions with respect to the baseline condition), it is unlikely that activations attributed to memory load may reflect trial repetitions.

Conclusions

A network of load–responsive regions was identified in a visuospatial paired-associates learning task that parametrically varied mnemonic load while controlling for performance and individual variations to increasing TD across subjects. On the 100% successful retrieval attempt, linear signal intensity changes with increasing TD were located in the right precuneus during encoding and in the middle occipital gyrus bilaterally, left superior occipital gyrus, left fusiform gyrus, bilateral cerebellum, right precuneus, and anterior cingulate gyrus during retrieval. A boxcar (zero-order) function better characterized such changes in activation within occipital–parietal, middle and inferior frontal gyri, and anterior cingulate regions during encoding and, as such, may represent a modulation of TD responses by task-specific processing demands and performance. Nonlinear load dependency, though not demonstrated with parametric analyses but with categorical analyses, resembled the BOLD response in right DLPFC and left inferior frontal gyrus. Greater activation of the *same* occipital–parietal, cerebellar and anterior cingulate regions as load increased during retrieval (and constant activation of the same regions during the easier task of encoding) suggests that increasing TD is supported by an increase in activation within the same, rather than an additional, network of brain areas.

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